

Pathophysiological and Clinical Importance of Insulin-Like Growth Factor-I with Respect to Bone Metabolism

I. ŽOFKOVÁ

Institute of Endocrinology, Prague, Czech Republic

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Summary

The modern concept of causality of diseases emphasizes the study of natural defense functions of the organism and possibilities of influencing them, which will lead to effective prevention of these diseases. A great deal of information has been obtained on the system growth hormone (GH)/insulin-like growth factor (IGF)-I, which is of quite fundamental importance for the integrity of the organism. A dysbalance of the system may be the cause of diseases of the neonatal period, as well as diseases associated with aging. In old age, the synthesis of the crucial peptide system, IGF-I, declines as well as the sensitivity of tissues to this hormone. At the same time the changes in the expression of IGF-binding proteins (IGFBP) occur. Systemic growth factors are present in measurable concentrations in the circulation, they are, however, taken up or synthesized by some tissues, where they act as local cellular regulators. IGF-I is produced by many tissues, including bones under the control of estrogens, growth hormone and the parathyroid hormone. A decline of bone IGF-I in the cortical portion of bones is one of the many mechanisms leading to the development of involutional osteoporosis. Correlation studies, which have provided evidence of a relationship between the IGF system and the building of peak bone mass and its subsequent loss contributed to the understanding of the pathogenesis of this disease. It may be foreseen that the results of intervention studies focused on the effects of the recombinant IGF-I will also influence therapeutic and preventive approaches. Modern antiresorption pharmacotherapy stabilizes or enhances bone density and reduces the risk of fractures. The addition of effective anabolics might increase the effectiveness of treatment by shifting the remodeling equilibrium in favor of formative processes. Because both recombinant GH and IGF-I have certain therapeutic limitations, it is considered to utilize substances which either stimulate endogenous IGF-I production directly in the bone or modulate synthesis and distribution of binding proteins for the peptide. Further new findings related to physiology and pathophysiology of this peptide will contribute to designing new strategies in the prevention of osteoporosis and other serious diseases of old age, such as diabetes, neoplasias or cardiovascular diseases.

Key words

Insulin-like growth factor-I • Growth • Development • Metabolism • Bone mineral density

Growth factors represent remarkable substances. The most important ubiquitous growth factors are the insulin-like growth factor-I and -II (IGF-I, IGF-II). The

hypothesis on the existence of IGFs was expressed by the end of the fifties, when Salmon and Daughaday (1957) provided evidence that the growth hormone (GH) does

not influence cartilage directly, but *via* an undefined factor of hypophyseal origin. Due to the availability of sensitive and specific methods, the bioactivity of IGF is being studied at present in a complex of other modulating factors, mostly in conjunction with aging. While IGF-II is mainly involved in the control of prenatal growth, IGF-I stimulates linear growth during the postnatal period. It is assumed that extreme gene expression of IGF-II could be the cause of some inborn growth disorders such as the Beckwith-Widemann syndrome. Despite the high plasma concentrations of IGF-II (roughly fourfold as compared with IGF-I), only a few findings on the metabolic and growth function of this peptide have been reported. Hence, so far the attention has been focused mainly on IGF-I, especially in relation to neoplasias, glycoregulation and bone metabolism.

GH – IGF system

Growth hormone (GH) is a peptide released from the pituitary gland episodically, following a circadian rhythm. Apart from stress, the principal stimulating factor of GH is the pulsatile secretion of hypothalamic peptide GH-releasing hormone (GHRH). The effect of a single injection of GHRH is almost completely specific for GH secretion (Reichlin *et al.* 1974). Most men older than 40 years have low or absent responses to GHRH (Shibasaki *et al.* 1984). Thus, the response of somatotrops to GHRH is age-dependent. GHRH exerts its effect *via* calcium signaling, as well as through cAMP or the phosphatidylinositol cycle (Brazeau *et al.* 1982). Furthermore, GHRH stimulates transcription of specific GH mRNA. The effects of the GHRH on GH secretion is potentiated by estrogen (Dawson-Hughes *et al.* 1986) and by glucocorticoids (Wehrenberg *et al.* 1983) and is strongly inhibited by somatostatin (Krush *et al.* 1968).

IGF-I is a peptide with a molecular weight of 7.0-7.5 kDa. The sequence of amino acids is very similar to that of IGF-II and proinsulin. The synthesis of IGF-I is controlled by a number of endogenous (genetic and hormonal) and exogenous (nutrition and physical activity) factors. The peptide is produced by a number of tissues, including adipose tissue (Wabitsch *et al.* 2000), but its main source is the liver, where the dominating regulators of IGF-I synthesis are insulin (independent predictor) (Hong *et al.* 1997) and growth hormone (Sjogren *et al.* 1999). While the GH concentration pulsates in the course of the day, the IGF-I level is stable (with the exception of acromegaly) (Skjaerbaek *et al.*

2000). Therefore, circulating IGF-I is considered an integrative indicator of GH activity and is used for monitoring the growth activity in children with deficient GH secretion. It was, however, found that IGF-I is not a sufficiently sensitive and specific indicator of GH deficiency in adults (Rosen 1999).

The acute fluctuations of IGF-I levels are controlled by insulin and by hepatic IGFBP-1 (Rosen 1997). Insulin shortage in diabetes mellitus type 1 is characterized by low IGF-I levels, although the GH level is high as a rule.

Further factors are involved in the control of homeostasis of the IGF-I system. IGF-I production is significantly stimulated by sex hormones. Serum IGF-I, levels in childhood, similarly as GH levels, are very low and they rise during puberty. Later, the IGF-I levels decline very slowly. During puberty, besides testosterone and estradiol, a positive effect on IGF-I synthesis is also exerted by the adrenal androgen dehydroepiandrosterone (DHEA).

Circulating IGF-I fluctuates in women during the menstrual cycle, the maximum values having been recorded in the late follicular and early luteal stage, probably as a result of fluctuations of estrogen levels (Helle *et al.* 1998). Soon after ovariectomy, a significant decline occurs following a transient rise of IGF-I levels (Žofková *et al.* 1996, Verhaeghe *et al.* 1998). Conversely, long-term estrogen substitution (extending over 5 years) reduces the activity of the IGF-I system (Vestergaard *et al.* 1999).

Although estradiol concentrations also correlate with IGF-I levels in men (Rosen 1999), a more marked relationship of the system has been observed with androgens. This fact explains lower IGF-I levels in women with GH deficiency as compared with similarly affected men (Garnero *et al.* 1999, Fisker *et al.* 1999). The predominating effect of estrogens or androgens on IGF-I production cannot be accurately defined due to the tissue conversion of androgens to estrogens. The drop of IGF serum levels in old age is, apart from other influences, the direct consequence of a reduced production of DHEA (Haden *et al.* 2000).

In the osseous tissue, the production of IGF-I is, in addition to estrogen, stimulated by the parathyroid hormone (PTH), which also stimulates the transcription of IGFBP-1 in osteoblasts and thus amplifies the osteotrophic effect of IGF-I (Pereira and Canalis 1999). The production of both IGF-I and IGF-II in human osteoblasts is also stimulated by 1,25(OH)₂ vitamin D₃

(1,25(OH)₂D₃ and calcitonin (Farley *et al.* 2000) or the calcitonin-gene-related peptide (Ballica *et al.* 1999).

A modulating effect on IGF-I production is also exerted by the transforming growth factor β , glucocorticoids and thyroid hormones (Inzucchi and Robbins 1996). The latter hormones increase the IGF-I production independently of GH, but also the activity of inhibitory IGFBP so that this anabolic effect may be cancelled (Inukai *et al.* 1999, Huang *et al.* 2000, Lakatos *et al.* 2000).

An investigation of monozygotic twins revealed that in addition to genetic factors about one third to one quarter of IGF-I, IGFBP-1 and insulin variations are determined by factors of the external environment such as nutrition or physical activity. The amount and quality of dietary proteins have an impact not only on the IGF-I concentration, but also on the production of its binding proteins, which modulate the action of IGF-I on protein synthesis in the organism (Noguchi 2000).

An important influence on IGF-I production is exerted by the nutritional status (Hotta *et al.* 2000). Malnutrition reduces the IGF-I levels markedly, even when the GH concentrations are high. Conversely, a stimulating effect of parenterally administered amino acids on IGF-I levels was demonstrated in rats (Kee *et al.* 1999). The IGF-I secretion is positively influenced by trace elements, in particular zinc (Devine *et al.* 1998) and manganese (Clegg *et al.* 1998). An important modulator of the IGF system is also physical activity (Oxlund *et al.* 1998, Berman *et al.* 1999).

Racial differences in serum IGF-I have been observed. Higher IGF-I concentrations and lower IGFBP-I levels were found in African American girls than in Caucasian subjects (Wong *et al.* 1999).

Taken together, IGF synthesis is influenced in many tissues by a number of endogenous and exogenous mechanisms, whereby the basic regulating factors at the hepatic level are insulin, GH and nutrition, in bones sexual and calciotropic hormones.

Regulation of IGF homeostasis – the role of IGFBP

IGF-I forms with IGFBPs inactive complexes which are broken down by tissue and circulating proteases. This ensures the stability of diurnal levels of free (biologically available) IGF, which is an indirect integrative parameter of fluctuating GH levels (Rechler and Clemmons 1998, Ferry *et al.* 1999a,b). Under normal conditions, free IGF-I accounts only for 1 % of the total volume of the circulating hormone (Yu *et al.* 1999). The

growth, mitogenic and metabolic function of the IGF system in various tissues depends on IGF synthesis, activation of IGF receptors, IGFBP (1-6) production and their proteases and probably also on the activation of receptors for these binding proteins (Singh 2000).

More than 75 % of the circulating IGF-I forms a trimeric complex with IGFBP-3 and its acid-labile subunit (ALS). Most of the circulating IGF-I is bound with IGFBP-3. Only small fractions of IGF-I are bound to the other six binding proteins, the importance of which is not as yet clear. IGFBP-3, which has the largest molecule of the binding proteins, together with IGFBP-5, ALS and IGF forms a complex with a molecular weight of 150 kDa (Boisclair *et al.* 2001). For this reason, it penetrates through the capillary membranes only with difficulty. The other substantially smaller binding proteins (in a complex with IGF-I molecular weight of 30-50 kDa) penetrate through the capillaries, but IGF is less firmly bound. There are speculations on the link with further structurally related peptides (IGFBP-related proteins) (Hwa *et al.* 1999).

Although IGF-I is linked mainly to its own specific receptors, it also has a certain affinity for insulin receptors, where it activates, similarly to insulin, tyrosine kinase, phosphoinositol 3-kinase and MAP-kinase *via* Grb2-Sos, Ras and Raf. Insulin receptors of many tissues, including the adipose tissue, can thus be activated by IGF, although with a 10-100 times lower affinity (Froesch *et al.* 1996). Nevertheless, in some tissues quite independent hybrid receptors for both these hormones were detected. Moreover, the distribution of receptors for insulin in the mammalian organism differs somewhat from the distribution of receptors for IGF-I. While the classical target organ of insulin is the liver and adipose tissue, receptors for IGF-I are not present in these tissues. Conversely, muscular tissue possesses both these types of receptors.

IGFBPs are believed to have direct tissue effects independent of IGF, probably involving their link not only to receptors for IGF, but also to their own, as yet unidentified receptors. Evidence has been provided that IGFBP-3 directly induces apoptosis in neoplastic tissues such as the mammary gland or prostate. A positive effect of this binding protein was also observed in the bone. IGFBP markedly enhanced bone formation in oophorectomized mice by stimulation of osteoblasts (Andress 2001). Moreover, IGFBPs possess a nuclear effect by which they control gene expression. The antiproliferative effect of IGFBP-3 is explained by a link with the nucleus and by interaction with the receptor for

retinoid (Baxter 2001). IGFBP-3 and IGFBP-5 exert their influence by reacting with viral oncoprotein cellular proliferation, apoptosis and malignant transformation (Ferry *et al.* 1999a). It is likely that the direct effect of IGFBPs (independent of the bond with IGF) modulates cellular aging and growth activity (Conover 1997).

The most important binding protein is IGFBP-3. Together with its ALS it is synthesized in the liver and endothelia under the control of somatotropin and IGF-I (Froesch *et al.* 1996). Despite the fact that chronic diseases and malnutrition markedly reduce IGF-I production, the concentration of IGFBP-3 remains relatively stable under these conditions. However, reduced IGFBP-3 levels have been found in children with somatotropin deficiency and a definite imbalance of IGFBPs was observed in patients with osteoporosis.

IGFBPs can have inhibitory as well as stimulating effects on the function of IGF-I (Baxter 2000, Yu and Rohan 2000). While IGFBP-1 and IGFBP-5 enhance the bond of IGF-I to receptors and thus stimulate their activity, IGFBP-3 inhibits this process (Ricort and Binoux 2001). The latter peptide can, however, enhance the IGF-I activity by transferring the peptide to the cellular nucleus (Ferry *et al.* 1999a).

The action of IGFBPs depends partly on whether it is systemic or local. Evidence was provided that local IGFBP-4 administration inhibits bone formation, but after a systemic administration the effect of this protein is stimulating (Miyakoshi *et al.* 1999).

The most important functions of IGFBPs (according to Conover 1997) are as follows

- a) Control of systemic and tissue IGF reserves
- b) Conformation of receptors for IGF and modulation of the bond of the peptide with receptors
- c) Prevention of hypoglycemia induced by IGF
- d) Regulation of the transfer of IGF between the intra- and extravascular space
- e) Prolongation of the half-life of IGF in the circulation
- f) Amplification of the IGF action by the formation of a readily available IGF pool or reduction of the biological availability of the peptide
- g) Activation of IGF-like receptors
- h) Modulation of tissue growth activity in old age

In addition to IGF and IGFBP proteases determine the function of the system breaking down the IGF-IGFBP complex. The function of proteases is possessed by kallikreins, cathepsin and metalloproteinases. They affect not only the systemic homeostasis of IGF, but also its autocrine and paracrine function (growth of ovarian follicles, activation of

osteoblasts and dermal fibroblasts, tumor growth). The protease activity is similarly to IGFBP expression modulated by a feedback with IGF (Ferry *et al.* 1999b). Therefore, assessment of all IGF components extends the information on the activity of the system.

Physiological role of IGF-I

Proliferative and antiapoptotic effects of IGF-I

In addition to its proliferative effect, IGF-I inhibits programmed cellular death – apoptosis. An anti-apoptotic effect of the IGF and IGFBPs was proved in cell cultures of some tumors (Granerus *et al.* 2001, Butt *et al.* 1999). Moreover, IGF-I interferes positively with reparative processes. Local IGF-I production in the kidneys during acute renal failure probably plays an important role in the repair of renal functions (Nishiki *et al.* 1999). The autocrine and paracrine action of IGF is also assumed in the healing of mucosal lesions in the intestine including post-resection lesions (Wiren *et al.* 1998). All components of the axis GH – IGF-I are expressed in immune cells, which explains the favorable influence of the system on immunity. IGF protects T-lymphocytes against apoptosis and together with GH has the function of a local differentiating factor – cytokine. The immunological effect of this system is particularly important during childhood (Yang *et al.* 1999). As receptors for IGF-I are present not only in leukocytes, but also in erythrocytes (Janssen *et al.* 1998b), IGF-I plays an important role in the control of erythropoiesis (Shih *et al.* 1999).

Along with other growth factors IGF-I acts in the cardiovascular system by suppressing apoptosis of cardiomyocytes and improving the cardiac output. By its action on the vascular endothelium it reduces pulmonary resistance (Lee *et al.* 1999a). The expression of IGFBP-3 mRNA in the myocardium strengthens the hypothesis on the direct effect of IGFBP-3 at the local level (Granata *et al.* 2000).

Metabolic effects of IGF-I

IGF-I is an anabolic hormone stimulating proteosynthesis, mainly in muscles. The catabolic action of tumor necrosis factor (TNF- α) interferes with this effect (Frost *et al.* 1997). In type 1 diabetics IGF-I reduces the blood sugar level. Although it has only 6 % of the hypoglycemic activity of insulin, the glycoregulating importance of the peptide is due to its high concentration in the bloodstream, which is 1000 times higher than the insulin concentration. Major fluctuations of the blood sugar level are prevented by the

bond of IGF with IGFBP. In insulin-resistant subjects and in type 2 diabetics IGF improves the sensitivity of muscles and liver to insulin, reduces the insulin consumption and the endogenous glucose production (Cusi and DeFronzo 2000). Moreover, IGF-I is an important paracrine regulator of adipogenesis (Marques *et al.* 2000).

The primary site of endocrine action of IGF-I is the hypothalamus, where IGF-I is produced but also taken up from the circulation, it stimulates somatostatin synthesis and inhibits GHRF (growth hormone releasing factor) production (Span *et al.* 1999). Another paracrine/autocrine source of IGF-I is the adenopituitary, where the peptide inhibits the transcription of GH by a feedback mechanism. The integrity of receptors for IGF-I on somatotropes is the prerequisite for the GH response to physiological stimuli such as GHRH, ghrelin, leptin and nutrition (Pombo *et al.* 2001). IGF-I together with insulin induce the gene transcription of prolactin. The physiological importance of IGF-I for control of prolactin secretion *in vivo* has, however, not been elucidated so far (for review see Melmed 1999).

IGF-I stimulates the gonadotropin secretion, in particular of the luteotropic hormone (Adam *et al.* 2000), amplifies the response of their receptors in the cells of the granulosa and enhances sex hormone synthesis (Hirakawa *et al.* 1999). Probably it ensures the harmony of puberty and growth (Huang *et al.* 1998). The stimulation of gonadotropins can partly explain the cancerogenic effect of IGF in some hormone-dependent tissues (prostate and mammary gland) (Holly *et al.* 1999).

The local IGF-I system in gonads participates in the auto- and paracrine regulation of steroidogenesis (Lallemand *et al.* 1998). In men the circulating IGF-I correlates markedly with the testosterone level and it is probable that testosterone directly stimulates IGF-I production (Jorgensen *et al.* 1998). In men older than 50 years, a correlation between all components of the system (IGF-I, IGF-II and IGFBP-3) and sex hormone binding protein has been proved (Pfeilschifter *et al.* 1996). Moreover, IGF-I probably has an impact on reproduction because a receptor for IGF-I has been detected in the human sperm cell (Naz and Padman 1999).

Clinical manifestations of impaired function of the IGF system

IGF-I deficiency

While untreated inadequate insulin secretion or defect of the insulin receptor are conditions incompatible

with life, extremely low IGF-I serum concentrations do not threaten vital functions, but are the cause of small stature. Conversely, a defect of the receptor for IGF-I (a deletion on the 15th chromosome) prevents normal tissue differentiation to such an extent that the deviation is not compatible with life (Froesch *et al.* 1996). Despite the fact that receptors for IGF-I are partly homologous with receptors for insulin, a defect in the expression of one of the two hormones or their receptors is manifested by a well defined effect. A physiological decline of activity of the somatotropin – IGF-I axis in all components including IGFBP-3 occurs during aging, when the function of the hypothalamo-pituitary system is reduced and the activity of sex hormones declines. In old age, protein malnutrition participates in the IGF-I system deficiency (Ponzer *et al.* 1999, Ammann *et al.* 2000) and vitamin D deficiency (Rosen and Conover 1997).

Low IGF-I levels as a result of primary resistance to GH characterize Laron's syndrome (Laron 1999). Moreover, insensitivity of IGF-I receptors is probably the cause of small stature in girls with Turner's syndrome (Lebl *et al.* 2001). Disorders of the IGF-I system can be recorded in some diseases. Very low serum IGF-I levels with low IGFBP are found in labile patients with type 1 diabetes, even when the somatotropin levels are high (Day *et al.* 1998). It is assumed that a low IGF-I level in these patients could be the cause of diabetic neuropathy. In type 2 diabetics the IGF-I activity is usually reduced as a manifestation of genetically coded insulin resistance (Froesch *et al.* 1996), but partly as a result of high concentrations of inhibitory IGFBP-1.

Both circulating IGF and IGFBP-3 decline rapidly with developing hepatic insufficiency (Schalch *et al.* 1998, Cemborain *et al.* 2000). Moreover, circulating IGFBP-3 has shown a better indicator of the hepatic synthetic capacity than serum albumin in patients with liver cirrhosis (Šídlová *et al.* 2002). Retarded growth in patients with chronic renal failure may be caused to a considerable extent by a low concentration of free IGF-I, but in part also by a resistance to somatotropin and IGF-I (Frystyk *et al.* 1999b). In addition, resistance to IGF-I and to GH is given as an explanation of osteodystrophy associated with some renal diseases and chronic metabolic acidosis (Green and Maor 2000). Impaired growth of patients treated with glucocorticoids can be explained by inhibited expression of the IGF system in the skeleton (Canalis 1998). Reduced autocrine and paracrine IGF-I activity in bone is probably one of the pathogenetic factors of osteoporosis.

IGF deficiency may participate in the development of some cardiovascular diseases. A

genetically determined low IGF-I serum level signalizes a greater risk of myocardial infarction (Vaessen *et al.* 2001). IGF-I deficiency causes delayed reparative processes including wound healing. An association with some neurodegenerative diseases is assumed, as well as with impaired cognitive functions (Aleman *et al.* 1999) and with reduced muscle strength (Kostka *et al.* 2000). Low IGF-I levels are recorded in critical states of systemic diseases, in particular those where cytokines are activated (Gelato 2000, Ng *et al.* 2000).

Increased activity of the IGF system

Physiologically elevated free IGF-I serum levels as a reflection of enhanced bone formation are recorded during periods of accelerated growth and puberty (Kawai *et al.* 1999). Pathologically raised IGF-I (and IGF-II) levels in the complex with IGFBP-3 are found in blood and cortical bone in acromegaly. IGF-I may play a role in the development of some diabetic complications: the peptide stimulates angiogenesis and thus leads to the development of retinopathy (Spranger *et al.* 2000). Similarly, a relationship was observed between the IGF system and microalbuminuric nephropathy (Cummings *et al.* 1998, Feldmann *et al.* 2000). Additionally, an imbalance of IGF activity with high levels of free IGF-I in non-diabetic subjects and low levels in type 2 diabetics is frequently associated with obesity (Frystyk 1999c).

Correlation analysis revealed that IGF-I could be important in the development of some hyperandrogenic states such as premature adrenarche or the polycystic ovaries syndrome (Vugin *et al.* 1999). A high circulating IGF-I level has been observed in estrogen-treated postmenopausal women, especially in those with very low initial IGF-I values (Posaci *et al.* 2001).

Even a mild elevation of IGF-I serum levels may exert a cancerogenic effect (Rosen 2000), which is amplified by low IGFBP-3 levels. In this respect, IGFBP-3 is thus a significant protector (Yu *et al.* 1998, Holly *et al.* 1999, Giovannucci 1999). High IGF-I levels, in particular if associated with a low IGFBP-3 concentration, involve a greater risk of colorectal carcinoma, breast cancer (in particular during the premenopausal period) and prostate cancer, where the relationship to the prostate seems to be quite independent of prostatic antigen levels (Hankinson *et al.* 1998, Tamada *et al.* 2001). The incidence of neoplasms of the prostate and mammary gland in relation to IGF-I expression is explained, in addition to the direct proliferative and antiapoptotic action of the peptide, by

stimulation of sex hormones and inhibitors of apoptosis e.g. interleukins or PDGF (DiGiovani *et al.* 2000). The role of the IGF system for the development of these neoplasms is supported by a more frequent incidence of carcinoma of the prostate and large intestine in acromegalic patients (Jenkins *et al.* 2000). An increased expression of the gene for IGF-I was proved in hormonally active adrenocortical carcinomas and in virilizing adenomas (Voutilainen 1998, Arnaldi *et al.* 2000).

The stimulating effect of a high energy diet on the growth of carcinomas can be explained *via* the IGF system (Yu and Rohan 2000). Conversely, the cytostatic effects of the retinoid fenretinide are explained through IGF-I – the latter reduces IGF-I production and raises the IGFBP-3 concentration. Moreover, the favorable anticancerogenic effect of dietary restriction is well known (Rosen 1999).

Some authors have emphasized the association of the system with benign prostate hyperplasia (Finne *et al.* 2000). It is assumed that IGF-I is of pathogenetic importance in simple goitre, the growth of which is stimulated directly, but also by enhancing the mitogenic activity of thyrotropin (Deleu *et al.* 1999). Moreover, the assessment of high IGF levels can assist in early diagnosis of some mesenchymal tumors associated with hypoglycemia and producing excessive amounts of IGF-II, which is bound to insulin, as well as IGF-I receptor (“big IGF-II”). Insulin and growth hormone secretion is usually suppressed in these patients due to feedback inhibition at the somatotrophic level and the B-cell level (Froesch *et al.* 1996).

The important factor determining the cancerogenesis of IGF concerns the activity of its tissue receptors. The large number of receptors for IGF-I in experimental animals was associated with a higher incidence of colorectal carcinoma (Hakam *et al.* 1999).

It may be summarized that the IGF system participates in the control of pathophysiological mechanisms of growth, as well as of cellular death by an important autocrine participation of ligands for receptors, binding proteins, proteases and receptors in a given tissue (Lackey *et al.* 2000, Khandwala *et al.* 2000). The pathogenetic importance of IGF-I for the development of neoplastic diseases is beyond doubt. Nevertheless, the possibility arises to use methods for the estimation of these individual constituents of the system in the serum and/or tissues for the early diagnosis and prevention of tumors.

Diagnostic use of IGF-I

The assessment of serum levels of free IGF-I (after previous radioimmunometric extraction by the "sandwich" technique and two monoclonal antibodies) has made it possible to use this parameter in clinical practice. In addition, the diagnostic application of IGF-I can be used for assessing the peptide concentration in urine (Hall *et al.* 1999). Low serum IGF-I concentrations are usually found in GH deficiency in children (less significantly in adults). The IGF-I response to somatotropin differentiates growth disorders into IGF-dependent and IGF-independent (Likitmaskul *et al.* 1998). The rise of circulating IGF-I is a good criterion of the effectivity of substitution treatment in affected children (Hilding *et al.* 1999, Murray *et al.* 2000). IGF-I, along with IGFBP-3, provides information on the activity of acromegaly (Rosen 1999, Span *et al.* 1999, Chen and Lin 1999).

Serum IGF-I levels are a reliable indicator of the nutritional status of the organism and correlate significantly with body mass index (Delcaya *et al.* 2000). Restriction of essential amino acids leads to a decline of IGF-I production and to subsequent growth retardation in children (Takenaka *et al.* 2000). Moreover, IGF-I serum levels provide information on the general status in diarrhoeal diseases, especially the coeliac disease (Locuratolo *et al.* 1999). Conversely, a rise of IGF-I level in these patients is a reliable indicator of the state of reconditioning (Bhutta *et al.* 1999).

Higher total IGF-I levels in the early stages of myocardial infarction indicate a satisfactory remodeling of the myocardium and restoration of ventricular function (Lee *et al.* 1999a,b). Low IGF-I levels in infertile men may signalize inadequate spermatogenesis which are frequently of autoimmune origin (Colombo and Naz 1999). The question of a possible use of the IGF-I system in screening subjects with the risk of malignancy is being discussed.

Therapeutic use of IGF-I and its modulators

Synthesis of the recombinant form of IGF-I made the therapeutic use of this hormone possible. Inadequate growth in genetically conditioned insensitivity of receptors for GH or IGF-I (Camacho-Hubner *et al.* 1999) is considered an unequivocal indication for IGF-I administration. If treatment of these dysfunctions is started at an early age, normal growth may be achieved (Ranke *et al.* 1999a,b, Arnhold *et al.* 1999). IGF-I was also tested successfully in acquired growth disorders such as coeliac disease (Locuratolo *et al.* 1999).

An important indication for IGF-I treatment are some forms of diabetes. IGF-I enhances the expression of glucose carriers (GLUT 2 and GLUT 4), similarly to insulin (Asada *et al.* 1998). Therefore, in type 1 diabetics, where the IGF-I synthesis in the liver usually declines, substitution treatment reduces blood sugar levels and insulin consumption. IGF-I also has a favorable effect in different forms of insulin resistance, including genetically conditioned ones (mutation of the gene for the receptor or postreceptor action of insulin) (Froesch *et al.* 1996). It was successfully tested in women with polycystic ovaries, where hirsutism also regressed along with the improvement of metabolic balance. IGF-I has a positive effect on the nitrogen balance in severe catabolic conditions after operations, in burns and malnutrition.

In chronic renal failure and in the reparative stage of renal ischemia, IGF-I increases the glomerular filtration and renal blood flow (probably *via* activation of nitric oxide) (Vijayan *et al.* 1999). The peptide stimulates the growth of cardiac muscle cells, improves the cardiac output and reduces peripheral vascular resistance. It has a protective effect on ischemic cerebral tissue (Wang *et al.* 2001).

Hitherto, a positive effect of recombinant IGF on healing of gastric lesions has been reported only in animals (Korolkiewicz *et al.* 2000) and on mucosal radiation changes (Mylonas *et al.* 2000). Along with IGFBP-1 and IGFBP-3 it has a favorable effect on wound healing. IGF-I administered to rats with GH deficiency improves the motility and morphology of immature spermatozoa and positively influences the reproduction (Vickers *et al.* 1999).

IGF-I in small doses acts favorably on processes of aging, the menopause and on the regulation of body weight. IGF-I has a positive effect on immune processes as well as human mental abilities. Additionally, it is probable that it will prove to be useful in the treatment of neurodegenerative diseases including Alzheimer's disease. The use of IGF in the treatment of senile osteoporosis is mentioned below. Inhibition of the IGF system is necessary in some diseases. There is a possibility of treating selected neoplasms using IGFBP-3 or analogues of somatostatin.

Factors limiting the therapeutic use of IGF-I

IGF-I treatment has its limitations. The practical impact of serious sequelae of long-term administration of growth factors, i.e. especially of all neoplasms, has not been confirmed so far. During IGF treatment, undesirable metabolic manifestations may develop – hypoglycemia

(in particular after large intravenous doses) and hypophosphatemia with subsequent hypotension. A more frequent incidence of gynecomastia was also observed.

The therapeutic dose of IGF-I has not been defined so far. Pharmacokinetic investigations revealed that in the case of administration of small, and thus from the aspect of side-effects, safe doses of recombinant IGF-I, when the physiological concentrations of the hormone are not surpassed in the circulation, the clinical effectiveness of the peptide is controversial. Moreover, with regard to individual variations of the binding capacity of tissues, the clearance and distribution of IGF-I (Frystyk *et al.* 1999a, Rosen 1999), it should be used on a strictly individual basis as to the dosage of the peptide. Safe treatment of the above mentioned diseases will only be possible after elucidation of the molecular basis of the action of IGF-I in context with other growth factors and binding proteins (Bach 1999).

Importance of IGF-I in the physiology of bone

IGF-I in cooperation with other growth factors, such as transforming growth factor β , fibroblast growth factor, prostaglandins from group E_2 and cytokines, plays a significant role in the control of the remodeling process of bone and has a favorable impact on the healing of fractures (Canalis 1991). Systemic factors are involved, which are taken up by the osseous tissue as well as local factors with an autocrine/paracrine function, produced by osteoblasts (Žák 1994a,b). It is believed that inadequate synthesis of growth factors may be one of the causes of osteoporosis.

The greatest attention in relation to the bone tissue has been devoted to the GH – IGF-I system. It is assumed that the pubertal increment of bone mass is genetically coded in part *via* IGF-I, which concurrently occurs with the maximal increase in the levels of this peptide (Rosen and Donahue 1998). However, bone formation is already activated some two years before the IGF-I peak, i.e. between the age of 11 and 12 years. Thus, IGF-I during this period is not the major stimulator of bone formation (Sabatier *et al.* 1996). By the age of 60 years, serum IGF-I levels decline roughly by 60 % in both sexes (Nicolas *et al.* 1994) concurrently with the sensitivity of osteoblasts to this peptide (Pfeilschifter and Ziegler 1998). These changes can be an important pathogenetic component of involutional osteoporosis (Boonen *et al.* 1997a,b).

There is a dynamic equilibrium between circulating IGF-I and peptide production in tissues.

However, the interpretation of changes of serum IGF-I levels as an indicator of local synthesis is very difficult, as IGF-I production by participating tissues is controlled by their own mechanisms. That is why the bone fraction of this peptide participates to a minor degree in the serum levels (Canalis 1997). Particularly, during the period of the menopause, the systemic values usually do not correspond to the peptide content of bones (Zhao *et al.* 2000).

Despite the positive effect of IGF-I on bones investigated which have shown better healing of fractures in animals treated with IGF-I (Schmidmaier *et al.* 2001), a number of clinical studies concerned with the relationship of bone density and serum peptide levels have not reported positive results (Gilberg *et al.* 2001). The causality of the relationship between circulating IGF-I and osteoporosis has not been confirmed so far, since the bone density in old age declines for many other reasons. The pathogenetic importance of the circulating IGF-I in the development of osteoporosis is supported rather by studies involving younger subjects, focused on gene polymorphism of IGF-I, which determines about 20 % of systemic IGF-I levels (Rosen 1999).

The IGF-I, similarly to other growth factors acts on bone directly. After binding to osteoblastic receptors it stimulates the expression of mRNA procollagen type I and collagen synthesis (Tanaka *et al.* 1994, 1998, Canalis 1997). Moreover, IGF-I inhibits collagen degradation by inhibiting the activity of osteoblastic collagenase (Canalis 1997). The activated osteoformation is characterized by an increased local production of IGF-I and some binding proteins (IGFBP-5) with subsequent stimulation of further osteoblasts and enhanced apposition of bone matrix (Canalis 1997, Zhao *et al.* 2000). In addition, IGF-I acidifies the medium between osteogenic cells and the bone matrix and thus improves the conditions for bone mineralization (Santhanagopal and Dixon 1999). Similarly to somatotropin it hastens bone remodeling by direct action on receptors in osteoclasts (Hou *et al.* 1997, Chihara and Sugimoto 1997).

IGF-I *mediates*, in addition to GH and insulin, the anabolic action of various osteotropic hormones such as the sex hormones, (Inzucchi and Robbins 1996). The relationship between IGF-I and DHEA levels partly explains the age-dependent diminution of bone mass due to the decline of IGF-I (Haden *et al.* 2000). Through IGF-I action the positive osteotropic effect of calcitonin and calcitonin gene-related peptide (CGRP) can also be partly explained (Ballica *et al.* 1999), as well as that of small anabolic doses of PTH. Furthermore, the osteoprotective

effect of exercise is believed to be mediated in part by the IGF-I – IGFBP-3 mechanism (Bermon *et al.* 1999). Apart from that, IGF-I improves the general calcium balance in the organism by stimulating absorption of this ion in the gut. In a cross-sectional study Fatayerji *et al.* (2000) found that the decline of IGF-I in aging men has a negative impact on calcium absorption, which is even more significant than vitamin D deficiency.

Recombinant IGF-I raises serum levels of procollagen type I more significantly than the osteocalcin concentration. Because procollagen I is an indicator of the early stage of bone formation, while osteocalcin rather that of the late stage, it may be assumed that IGF-I activates especially the early stages of osteoblastic formation. Conversely, administration of growth hormone secretagogue – MK-677 – markedly increases osteocalcin levels concurrently with IGF-I levels and thus activates the late stage (Murphy *et al.* 1999). However, IGF-I stimulates bone remodeling as a whole, so that it thus hastens bone resorption.

The effects of IGF-I on bone can be assessed by means of bone remodeling markers. The decline of IGF-I levels with age is obviously the main cause of the slowing down of remodeling in old age, which is manifested by a decline of osteocalcin levels and N-telopeptide urinary levels (Murphy *et al.* 1999). The relationship between IGF-I levels and bone remodeling is closer in young subjects than in old ones (Fatayerji and Eastell 1999).

The response of different stages of the bone metabolism to IGF-I is dose-dependent. As small doses mainly stimulate the osteoblastic function, while resorption is activated only to a minimal extent, the resulting effect on bone is anabolic (Ghiron *et al.* 1995). The superiority of the anabolic action of small doses of IGF-I is promising as regards to the therapeutic use of this substance. It has been reported that bone metabolism is influenced by other constituents of the IGF system, including binding proteins by their impact on biological availability of the peptide for bone tissue (Mohan and Baylink 1995). Recently a direct osteoforming effect of IGFBP-4 has been proved (Miyakoshi *et al.* 2001a,b). Because serum IGF-I levels correlate closely with bone markers, some authors ascribe the importance of an indicator of bone remodeling to this parameter (Danielsen and Flyvbjerg 1996, Kim *et al.* 1999, Naylor *et al.* 2000).

IGF-I also acts on bone indirectly by inhibiting the expression of receptors for PTH and for PTH-related peptide (PTHrp) in osteoblasts (Bosch 1999). In addition, IGF-I is a hormone that stimulates muscle protein

synthesis and impairs protein degradation (Fryburg 1994, Jurasinski and Vary 1995, Frost *et al.* 1997). The anabolic action of IGF-I on muscle protein synthesis builds up the muscle mass and increases its strength, e.g. in myopathies (Kanda *et al.* 2001), which is a well-known osteoprotective mechanism.

An important osteoinductive factor is insulin (Verhaeghe and Bouillon 1994, McCarty 1995). It is known that insulin deficiency leads to growth retardation in the uterus and after delivery. In this case the growth effect is not only mediated by IGF activation, but directly through insulin receptors in osteoblasts and/or signaling at the postreceptor level (Menon and Sperling 1996, Thomas *et al.* 1997). This is why the osteoblast function declines markedly in type 1 diabetics (Bouillon *et al.* 1995). The physiological importance of insulin in bone metabolism was documented by the results of our investigation, where we provided evidence that insulin is an independent predictor of bone remodeling in non-diabetic women (Žofková *et al.* 2001). The hypothesis about the positive effect of insulin on bone is supported by the recently published study confirming a positive effect of hyperinsulinism on bone density in girls with premature puberty where the effect of insulin is amplified by hyperandrogenism (Ibanez *et al.* 2000). The pathogenetic importance of inadequate insulin secretion for bone metabolism and the risk of development of osteoporosis in diabetic subjects will require further investigation.

Genetics of IGF-I and bone density

The heritability of circulating IGF-I has been proven in a population study (Comuzzie *et al.* 1996) and in a mother-daughter study (Kurland *et al.* 1998b). Rosen *et al.* (1998) have documented the association between IGF-I polymorphism (microsatellite 192/192) and serum IGF-I, but no relationship to bone density was found (Miyao *et al.* 1998). The complexity of the association between the gene and two phenotypes (serum IGF-I and bone mass) may be explained by the interplay with a number of other genes and environmental factors.

Relationship between systemic IGF-I and bone homeostasis

The relationship of IGF-I to bone mass is apparent throughout the life, starting with puberty and during the pre- and post-menopause (Calo *et al.* 2000). The main pathogenetic mechanism of involutional

osteoporosis consists of retarded proliferation and maturation of osteoblasts and impaired mineralization as a result of inadequate production of growth factors and a reduced sensitivity of bone tissue to these factors (Marie 1997, Conover 1997). Therefore, the functional insufficiency of the GH-IGF-I axis is of special importance for the loss of bone mass during aging. These facts have been confirmed by correlative studies as well as by studies documenting the association of IGF-I status with osteoporosis.

Correlative studies

A study on inbred rats with a low and high bone mass confirmed close correlations between serum IGF-I levels, alkaline phosphatase (ALP) and bone density (Rosen *et al.* 1997). The importance of circulating IGF-I for bone metabolism is supported by many clinical studies, which have proved correlation between serum IGF-I and bone density (Reed *et al.* 1995, Boonen *et al.* 1996b, Rosen and Donahue 1998, Langlois *et al.* 1998) or bioptic parameters (osteoblastic activity, volume and mineralization of the osteoid) (Reed *et al.* 1995). Positive relations of serum IGF-I levels to bone density of the lumbar spine, neck of the femur and total bone mass were proved in younger (under 60 years old) and older (above 70 years old) postmenopausal women (Boonen *et al.* 1996a, Kim *et al.* 1999). Higher IGF-I levels are also mentioned in connection with higher values of the bone mass in black women (Yanovski *et al.* 2000). In addition, the IGF-I/IGFBP-3 index has been found to be a good predictor of low bone mass at the neck of the femur in runners (Snow *et al.* 2000).

In men positive relationships between serum IGF-I and bone density at the lumbar spine (Janssen *et al.* 1998a) and at the neck of the femur (Gillberg *et al.* 2002) have been found. Similar results have been published by Kurland *et al.* (1998a), namely in the case of a normal secretory GH reserve, which stresses the importance of isolated IGF-I deficiency.

Association studies

Osteoporotic women had, in addition to significantly lower levels of both 25-OH vitamin D₃ and 1,25(OH)₂ D₃ and higher parathyroid hormone levels, lower circulating IGF-I (Landin-Wilhelmsen *et al.* 1999). The importance of the IGF-I/IGFBP-3 complex for bone density was supported by a study demonstrating low concentrations of both components of this complex in patients with idiopathic osteoporosis (Wuster *et al.* 1993).

Low IGF-I levels have also been found in some forms of secondary osteoporosis, e.g. in hyperthyroidism (Foldes *et al.* 1997), in viral cirrhosis of the liver (Gallego-Rojo *et al.* 1998), juvenile chronic arthritis (Woo 1994) and in beta thalassemia (Soliman *et al.* 1998). A lack of balance between components of the IGF system together with inadequate proinsulin synthesis may participate significantly in a more rapid loss of bone mass in type 1 diabetes, in which a decline in serum IGF-I, (and IGFBP-3) and a decrease of bone density at the spine and at the hip have been found (Jehle *et al.* 1998). Furthermore, some authors have emphasized importance of IGFBP-3, the levels of which were significantly lower in osteoporotic men than in controls (Wuster *et al.* 1993, Johansson *et al.* 1997, Ferry *et al.* 1999a, Gillberg *et al.* 2002).

Limitations of clinical studies on the association between circulating IGF-I and bone parameters

Threshold levels of circulating IGF-I are necessary for normal bone growth and density in animals, which suggests that the IGF-I system plays a prominent role in bone health (Yakar *et al.* 2002). However, a recently published paper focused on the relationship of systemic IGF-I and bone in humans brought controversial results (Pfeilschifter *et al.* 1996). As already mentioned, IGF-I and binding proteins are taken not only from the blood, but also produced in bones under the control of 1,25(OH)₂D₃, PTH and sex steroids (Ravn *et al.* 1995, Canalis 1997). While the pathogenetic importance of local IGF-I for the bone mass is beyond doubt, the importance of its serum levels is not quite unequivocal (Ravaglia *et al.* 2000). The secretory responses of IGF-I, IGF-II and IGFBP-3 to recombinant GH in osteoporotic women were comparable with the response of healthy women (Kassem *et al.* 1994). Moreover, Lloyd *et al.* (1996) did not find any correlation between serum IGF-I levels and the bone mass of the neck of the femur or lumbar spine in a group of peri- and postmenopausal women. Similarly, negative results were recorded by Landin-Wilhelmsen *et al.* (1999). The relationship of serum IGF-I to bone mass in healthy postmenopausal women was also not confirmed in a study where a correlation was proved between IGF-I and indicators of bone remodeling – osteocalcin and the bone isoenzyme ALP (Collins *et al.* 1998). We conducted a correlative cross sectional study in a group of 146 pre- and postmenopausal women. After adjustment for age, body mass index, serum PTH, calcitonin and 25-OH vitamin D, we did not find any relationship between serum IGF-I and

bone mass at the lumbar spine or at the hip, while 25-OH vitamin D and testosterone were good predictors of bone density (Žofková *et al.* 2001). Negative results, in regards to bone density and systemic IGF-I levels in elderly men, were obtained by Gurlek and Gedik (2001). In the study of Garnero *et al.* (2000) serum IGF-I concentration accounted for less than 6 % of the interindividual variability of bone density at different sites of the skeleton. Therefore, the authors assumed that the circulating peptide exerts only a small, though detectable effect on bone mass.

The results of research focused on investigation of the relationship between IGF-I serum levels and bone mass have important limitations. The measurement of the circulating IGF-I levels may underestimate the impact of IGF-I on bone metabolism. IGF-I produced locally in bone osteoblasts, which does not correlate with the circulating IGF-I, exerts its anabolic effect primarily by increasing the activity of resident osteoblasts (Zhao *et al.* 2000). Several studies have found a relationship between the concentration of IGF-I in the human skeleton and bone mass (Dequeker *et al.* 1993, Nicolas *et al.* 1994, Boonen *et al.* 1997a, Aersens *et al.* 1997, Seck *et al.* 1998). The latter study based on histomorphometric measurements documented that the bone matrix IGF-I was significantly associated with bone volume.

Furthermore, IGF-I may be assumed to have an impact not only on mineralization but also on the structural integrity of bone (Boonen *et al.* 1997b). The importance of the IGF-I system for bone quality is apparent from studies providing the evidence of a low IGF-I (and IGFBP-3) level in women with fractures, independently of the bone density. Women with low IGF-I levels had a 60 % higher incidence of fractures (Sugimoto *et al.* 1997, Hedstrom 1999, Garnero *et al.* 2000). Moreover, other components of the IGF system – binding proteins and proteases – as well as the sensitivity of bone tissue to IGF-I which decrease with advanced age, have not been assessed in the majority of the above mentioned studies (Pfeilschifter and Ziegler 1998). A modulating effect may be exerted by the interaction with many other humoral factors, the influence of which was not ruled out in these projects. Lastly, the factor of age is very important. Substantially closer correlations were observed between circulating IGF-I and bone density in subjects younger than 35 years old, i.e. during the peak period of bone mass, when the IGF-I levels are markedly higher than in women above this age (Ravn *et al.* 1995). Conversely, the majority of quoted investigations were made in postmenopausal or elderly populations. These

facts indicate the difficulties associated with the interpretation of the clinical results.

The significance of GH and/or IGF-I in the treatment of osteoporosis

Although both GH and IGF-I meet the prerequisites of effective bone anabolics, the research concerned with the therapeutic use of GH in patients with osteoporosis and normal hormone status has not hitherto provided very optimistic results. An unequivocally positive response to recombinant GH or IGF-I was recorded in adolescent osteoporotic subjects with deficient somatotrophic secretion before reaching the peak of bone mass (Underwood *et al.* 1999). On the other hand, the effect of these growth factors is uncertain in adults after the age of 60 years and in particular in advanced age with acquired GH deficiency (Brixen *et al.* 1998). Because somatotropin hastens bone remodeling, assessments of bone mass made during the first few months of treatment may even show a temporary decline of the values. Later, even in old people, the value of bone mass may slightly increase, but this increase is not comparable with the response of young subjects with organic GH deficiency. A further disadvantage is a very narrow therapeutic range when the effective doses of the hormone may be close to the borderline of tolerance. It was, however, found that the hormone could enhance the effect of osteotropic drugs such as calcitonin. Very perspective appear to be secretagogues of GH – GHRH (growth hormone releasing hormone) or GHRH analogues, which stimulate the endogenous GH axis by a pulsatile action thus affecting bones by a more physiological mechanism.

A new strategy in the prevention and treatment of osteoporosis involves stimulation of the GH axis by arginine, which activates osteoblasts and collagen synthesis (Chevalley *et al.* 1998). Moreover, L-arginine stimulates nitric oxide synthesis, the latter being an effective inhibitor of osteoclastic resorption (Visser and Hoekman 1994). In malnourished patients an adequate response of GH has been observed after administration of proteins (Schurch *et al.* 1998).

Bone formation can be stimulated by small doses of recombinant IGF-I with minimal activation of bone resorption. This substance can, therefore, be an important asset in the treatment and prevention of osteoporosis with a low bone metabolic turnover (during the somatopause or during glucocorticoid treatment), as well as in some forms of postmenopausal osteoporosis (Verhaeghe *et al.*

1996, Marcus 1997, Berneis *et al.* 1999). The beneficial effect of IGF-I on bone metabolism was recorded in patients with liver cirrhosis (Cemborain *et al.* 2000).

The advantage of IGF-I, in addition to the mineralization effect, is an improvement of bone geometry parameters and thus also the quality of bone tissue (Wuster *et al.* 1998). The positive effect on the skeleton is enhanced by its binding with IGFBP-3. The administration of recombinant IGF-I in a complex with IGFBP-3 markedly enhances bone density of the femur in ovariectomized rats (Bagi *et al.* 1995). Similarly, it was found that recombinant IGF-I in the complex with binding protein (rhIGF-I/IGFBP-5) enhances the growth of the cortical bone in the same species. It does not, however, influence trabecular bone (Bauss *et al.* 2001). The finding of a positive effect of IGFBP-4 is of interest. It increases the parameters of osteoformation after systemic administration, which is explained by the enhanced biological availability of IGF (Miyakoshi *et al.* 2001a).

To conclude, IGF-I is an extremely effective osteotropic growth factor. Serum concentrations of this peptide are relatively high, but its local biological

effectiveness is, similarly to other systems, modulated by its binding with IGFBPs, the activity of protease and tissue sensitivity. While circulating IGF-I levels can characterize to a great extent the state of bone metabolism, the problem of the predictive importance of systemic IGF-I for bone mass still remains open. Although the causality of the relationship between serum IGF-I levels and bone density cannot be proved, the pathogenetic role of local IGF-I is beyond doubt. The clinical importance of individual IGFBPs (Andress 2001) remains to be determined. The information to be derived from IGF I in relation to neoplasms and the risk of enhanced mortality has not been unequivocally resolved, although the relationship of high concentrations of the peptide to some tumors is obvious (Raynaud-Simon *et al.* 2001). Evidence of a linkage IGF-I gene polymorphism with neoplasms or osteoporosis would contribute to the elucidation of the pathogenesis of these serious diseases.

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Reprint requests

Doc. MUDr. Ivana Žofková, DrSc., Institute of Endocrinology, Národní 8, 116 94 Prague 1, Czech Republic. E-mail: izofko@endo.cz