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INSULIN-LIKE GROWTH FACTORS (IGFs) AND THEIR BINDING PROTEINS (IGFBPs) AS BIOMARKERS OF CATABOLISM

INSULINU SLIČNI FAKTORI RASTA (IGF) I NJIHOVI VEZUJUĆI PROTEINI (IGFBP) KAO BIOMARKERI KATABOLIZMA

Olgica Nedić[1], Judith Anna Nikolić[1], Vesna Malenković[2], Ivona Baričević-Jones[1]

[1]INEP – Institute for the Application of Nuclear Energy, University of Belgrade, Belgrade, Serbia
[2]Medical Centre »Bežanijska Kosa«, Belgrade, Serbia

Summary: The life of an organism depends on its capacity to maintain equilibrium. Disease induces acute adaptive responses specific to the stimulus and the organism achieves stability through change. These changes are associated with increased growth hormone resistance and activity of the hypothalamic-pituitary-adrenal axis, altered insulin response and cytokine synthesis and, closely related to all these events, alteration of the complex system interrelating insulin-like growth factors (IGFs), IGF-binding proteins (IGFBPs) and their receptors. This review aims to provide an overview of the results obtained in our laboratory from studies of the IGF system in patients with metabolic disturbances caused by infection, hepatobiliary and gastrointestinal diseases, open or laparoscopic surgery and postoperative sepsis.

Keywords: insulin-like growth factors (IGFs), IGF-binding proteins (IGFBPs), catabolism

Kratak sadržaj: Život zavisi od sposobnosti organizma da održi ravnotežu, prilagođavajući se u cilju postizanja stabilnog stanja. Bolest izaziva akutni odgovor kojim se organizam adaptira na stimulans. Promene se ispoljavaju kroz povećanu rezistenciju na hormon rasta, pojačanu aktivnost ose hipotalamus-hipofiza-nadbubrežne žlezde, izmenjen insulinski odgovor, kao i promenu u sintezi citokina. U bliskoj vezi sa ovim promenama je i promena u kompleksnom sistemu koji povezuje insulinu slične faktore rasta (IGF), IGF vezujuće proteine (IGFBP) i njihove receptore. Cilj ovog revijskog rada je da objedini rezultate do kojih je došla naša laboratorija proučavajući sistem IGF kod pacijenata sa metaboličkim promenama izazvanim infekcijom, hepatobilijarnim i gastrointestinalnim bolestima, laparoskopskom intervencijom, otvorenom operacijom i postoperativnom sepsom.

Ključne reči: insulinu slični faktori rasta (IGF), IGF vezujući proteini (IGFBP), katabolizam

Introduction

Growth hormone and insulin-like growth factors

There is a complex interplay in the metabolic actions of growth hormone (GH) and the insulin-like growth factors (IGFs). Thus, GH interacts with GH receptors, activating a complex signaling transduction cascade of events, including the generation of IGFs. There are many important metabolic functions of GH, some of which are mediated through IGFs, whereas others are not.

The IGF family consists of two peptides, approximately 7.5 kD in size, IGF-I and IGF-II, that share both structural and functional homology with insulin (1). Whereas insulin is generally thought to act mainly on metabolic processes, IGFs have both metabolic and mitogenic actions, regulating cellular proliferation and growth. Of the two, IGF-I is the principal anabolic regulator in post-natal life, while IGF-II plays a key role in fetal development (2). The biological actions of IGFs are mediated mainly by the type 1 IGF receptor (IGF-1R), which binds both peptides, and

Address for correspondence:
Olgica Nedić, PhD
INEP – Institute for the Application of Nuclear Energy
University of Belgrade
11080 Belgrade, Banatska 31b
Tel.: (+) 381 11 617 252
Fax: (+) 381 11 618 724
e-mail: olgica@inep.co.yu

the IGF-II/cation-independent mannose-6-phosphate receptor (IGF-2R) that strongly binds only IGF-II (3). When bound to this receptor, which has no known signal transduction function, IGF-II is internalised and degraded. The IGF-1R is a member of the tyrosine kinase receptor family.

As it is a protein-anabolic hormone, GH action usually leads to a significant increase in nitrogen retention and protein synthesis and a decrease in protein breakdown. The available data suggest that IGFs mediate the protein-anabolic actions of GH in man as they have similar pathways for enhancing protein anabolism (4).

Human adipocytes express GH receptors and GH has significant effects on fat metabolism. Thus, GH has been shown to decrease lipoprotein lipase activity, diminishing the flux of free fatty acids to the adipocytes, leading to a favourable lipid profile and reducing adiposity. IGF-I does not mediate the effects of GH on lipid/lipolysis and there are no functional IGF-1R in adipocytes (1).

In infancy GH is essential for normal glucose homeostasis and hepatic glucose production, but this critical role is markedly diminished in adults. The mechanisms that regulate the transition are poorly understood. However, IGF-I has very different effects on carbohydrate metabolism. These insulin-like actions of IGF-I are mediated mainly through IGF-1R, but IGF-I can also activate the insulin receptor, causing hypoglycaemia, although IGF-I has only 7.5 % equipotency with insulin (5). In general, IGF-I administration results in improved insulin sensitivity. Although the metabolic consequences of altered GH and IGF-I levels on insulin sensitivity and actions are known, the sequence of molecular events has not been clarified yet. Nevertheless, it has been demonstrated that at certain post-receptor levels the signals of GH and its main mediator, IGF-I, converge with that of insulin.

IGF-binding proteins and IGF/IGFBP complexes

Unlike insulin, the IGFs are bound with high affinity (Kd ~ 10^{-10} mol/L) to a family of six specific binding proteins, IGFBP-1 to -6 (6). Like the IGFs, the IGFBPs are produced in a variety of tissues and found in physiological fluids. All six IGFBPs form binary complexes with IGFs (40–50 kD), but IGFBP-3 and –5 also form ternary complexes (150 kD), together with an acid-labile subunit (ALS) produced in the liver. The synthesis of many IGFBPs, particularly IGFBP-3, and ALS is enhanced by GH. IGFBPs modulate (potentiate or inhibit) the growth-promoting and metabolic actions of IGFs but may have independent actions, e.g. platelet and nuclear uptake of IGFBP-3 which also has a glycosaminoglycan (heparin) binding site.

The tissue in which IGF-I expression normally correlates most closely and positively with GH levels is the liver. However, fasting induces a state of GH resistance characterised by GH hypersecretion and low circulating IGF-I levels (7). Fasting may also decrease the concentration of free IGF-I more rapidly by increasing the concentration of certain IGFBPs. Free IGF-I accounts for a minor fraction (less than 1 %) of the total circulating IGF-I, but only this form of IGF-I can elicit effects through IGF-1R. There are several studies indicating the importance of free versus total IGF-I for both short-term metabolic changes (during glucose tolerance testing) and long-term steady-state changes (linear growth). The specific IGFBPs directly regulate the amount of free IGF-I.

IGFBP-3 as regulator of IGF bioavailability

In healthy persons, about 90 % of the circulating IGFs are tightly bound to IGFBP-3, in a ternary complex. This high-molecular weight complex cannot pass through the capillary barrier and is, therefore, not available to the tissues. The complex is formed in the liver and is considered to be an IGF reservoir in the blood. Thus, the serum total IGF concentration is about two orders of magnitude higher than the insulin concentration. Determination of the relative molar ratio of total IGFs and IGFBP-3 may serve as an estimation of free IGF levels (8). In healthy adults the relative molar ratio of IGF-I + IGF-II and IGFBP-3 is close to 1. As the concentration of IGF-II is about three times greater than IGF-I, the molar ratio IGF-I : IGFBP-3 is approximately 0.25. This ratio is expected to be higher in persons with GH and IGF-I hypersecretion (in patients with pituitary tumors) and lower in children born small for gestational age or in persons with severe hepatic disease (liver dysfunction). The availability to the tissues of IGFs bound to IGFBP-3 can be increased by the action of various plasma proteases, as proteolysed IGFBP-3 has significantly reduced or no IGF affinity. Increased IGFBP-3 proteolytic activity was described in the serum of severely ill patients and in patients following surgery (9).

Metabolic regulation of IGFBP-1

IGFBP-1 differs from the other IGFBPs in its rapid regulation according to the metabolic state of the organism. Primarily synthesised in the liver by hepatocytes, the blood level shows diurnal variation with increased release between meals and suppression following food intake (10). In its marked response to hypoglycaemia, IGFBP-1 resembles the counterregulatory hormones, glucagon and cortisol, which can enhance its synthesis. It fulfills a counterregulatory role by blocking the insulin-like activity of IGFs. Insulin potently suppresses IGFBP-1 synthesis. The IGFBP-1 level may be greatly elevated on admission to the intensive care unit, possibly reflecting a stress-related rise in cortisol (10). Although the IGFBP-1

response to hypoglycaemia is seen even in patients who do not show a cortisol response, patients with chronic hypercortisolism have markedly depressed serum IGFBP-1 concentrations. This suppressive effect may be secondary to cortisol-induced hyperinsulinism. IGFBP-1 elevation in acute critical illness may also be caused by the stimulatory action of cytokines. The IGF-I : IGFBP-1 ratio has been shown to correlate with the insulin : glucagon ratio in burned patients (11).

Other IGFBPs

The roles of the other IGFBPs in the circulation are less well understood. IGFBP-2, generally the second most abundant specific binding protein, appears to be elevated under conditions where the total IGF-I + IGF-II concentration exceeds the capacity of IGFBP-3. The binary complexes can pass through capillary walls into the interstitial fluid, possibly making IGF more available to essential tissues. A number of disease states are characterised by elevated serum IGFBP-2 (9). Nutritional disturbances and cytokines may influence IGFBP-2 concentrations. Serum IGFBP-4 and -6 levels may also be elevated in critically ill patients. These two IGFBPs are GH-independent and their synthesis is probably regulated by a direct cytokine effect on the liver (12). In contrast, IGFBP-5 concentration is decreased in long-term critical illness (9). It is difficult to speculate on the consequences of these IGFBP changes in patients, in the absence of a complete understanding of the role of each in normal physiology. The increases in the IGFBPs that form only binary complexes with IGFs may provide a vehicle for increased IGF distribution to critical tissues. On the other hand, since most IGFBPs can impair IGF action by competing with the receptor for free IGF, their IGF uptake may serve to decrease IGF bioavailability.

Over the past decade, there has been growing awareness of the importance of the IGFs and their binding proteins in regulating growth and metabolism. Circulating concentrations of IGFs and IGFBPs change in response to the neuroendocrine and nutritional status of the organism and they are also influenced by inflammatory cytokines. Monitoring IGF-I, IGF-II, IGFBP-3 and IGFBP-1 concentrations may be informative as an index of patient responsiveness to stress. It is suggested that the levels of these substances are related to the hepatic insulin response, changes in nitrogen balance and, over the longer term, to predictiveness of the outcome. This review aims to provide an overview of the results obtained in our laboratory during studies of the IGF system in patients with metabolic disturbances. These were caused by infection, hepatobiliary and gastrointestinal diseases, open or laparoscopic surgery and postoperative sepsis.

Results and Discussion

Analytical aspects of IGF determination

As previously stated, IGF-I is a multifunctional mitogenic peptide, the main mediator of GH anabolic action in post-natal life, while IGF-II primarily functions as a growth factor during fetal development. The physiological function of IGF-II in adult man is poorly understood, although there is evidence of its involvement in mitosis, especially in pathological conditions, like tumor growth. Circulating concentrations of IGF-I and IGF-II do not show significant diurnal, gender or seasonal variations (up to several percentage units) (13). After peaking around puberty, mean serum IGF-I concentration declines with age in both sexes, while a significant fall in IGF-II with age was detected only in women (14). Characteristic IGF concentrations vary widely between individuals. A small rise in serum IGF-I concentration from the mid-follicular to the ovulatory phase of the menstrual cycle was detected. This is most likely mediated by estradiol action on GH secretion, as the IGF system has an important autocrine/paracrine role in ovarian follicle development (15, 16).

Each physiological fluid is a complex microenvironment in which IGF molecules are bound to specific combinations of IGFBPs. Thus, analytical assays

Table I The concentrations of IGF-I, IGF-II and IGFBP-3 in patients with metabolic disturbances.

Pathology/ Surgery	IGF-I concentration (nmol/L)	IGF-II concentration (nmol/L)	IGFBP-3 concentration (nmol/L)
None (healthy persons) N = 81	23.1 ± 7.99	72.1 ± 14.41	114 ± 29.5
Liver cirrhosis N = 65	13.4 ± 8.04[c]	35.6 ± 20.54c	–
Liver cyst(s) N = 143	14.4 ± 6.53[c]	66.0 ± 21.05a	–
Trichinellosis N = 20	18.4 ± 4.98[a]	67.6 ± 16.10	–
Gastrointestinal inflammation N = 92	16.1 ± 6.40[b]	61.3 ± 15.71[b]	63 ± 32.9[c]
Helicobacter pylori infection N = 122	13.4 ± 4.94[c]	67.2 ± 17.67[a]	–
Gastric tumors N = 25	15.0 ± 4.88[c]	65.9 ± 3.12[a]	88 ± 8.9[a]
Postoperative sepsis N = 25	7.3 ± 3.56[d]	69.0 ± 14.55	58 ± 19.1[c]
Cholecystitis N = 30	12.4 ± 5.81[c]	60.6 ± 15.00[b]	85 ± 20.4[b]

Data are shown as mean value ± SD. Statistically significant difference between healthy subjects and patients at $p < 0.05$(a), $p < 0.005$(b), $p < 0.0005$(c) and $p < 0.00005$(d), respectively.

for IGFs must take into account the profile of IGFBPs in each physiological milieu and the methods primarily designed for serum need adaptation for other samples, such as urine (17), milk (18) and seminal plasma (19).

In our laboratory, serum concentrations of IGF-I and IGF-II were measured by radioimmunoassay (RIA-IGF-I and RIA-IGF-II, INEP, Belgrade, Serbia) (20, 21). We have performed several studies that included patients with metabolic disturbances caused by different pathologies. An overview of the results is presented in *Table I*. Numerical data are expressed as the mean value and standard deviation (SD). Differences between the groups were analysed by the non-parametric Mann-Whitney U test.

IGFs and liver cirrhosis

More than 15 years ago, it was shown that peripheral serum concentrations of IGF-I and IGF-II decrease in liver diseases. Since then, investigations have been made on the possibility of using serum IGF-I levels as an early marker of liver dysfunction and/or predictor of survival in patients with cirrhosis. IGF-II has largely been ignored, even though earlier data suggested that differentiation of normal from pathological values might be more clear for IGF-II than for IGF-I. We conducted an investigation on a group of patients in order to evaluate the relevance of IGF-I and IGF-II determination in staging liver cirrhosis (22). The etiology of cirrhosis was recognised as chronic viral infection, excessive alcohol consumption, Wilson's disease, autoimmune and as of unknown origin. Serum levels of both peptides were found to be lower in patients than in age-matched healthy subjects. No correlation between disease severity (Child score) and serum IGF-I was observed, while IGF-II concentrations were correlated with Child score, suggesting that serum IGF-II concentrations may reflect compromised hepatic function more closely than IGF-I.

IGFs and parasitic infections

Pathophysiological cellular changes and physical obstruction due to increasing mass of a cyst may also compromise liver function. Cysts may be of different origin, affecting hepatic metabolism in various ways and to different degrees. Liver cysts in the adult can be classified as developmental, neoplastic, inflammatory, hydatid or miscellaneous (23). The patients involved in our study were divided into three groups according to the immuno-chemical reaction of their sera towards *Echinococcus granulosus* protoscoleces (24). Group I comprised samples that were typically sero-positive, confirming that *E. granulosus* was responsible for the cyst formation. Sera in group II exhibited specific immunofluorescent staining only on the protoscolex tegument. Thus they recognised antigens that are common for *E. granulosus* and other cross-reactive pathologies. Such cross-reactivity was shown in cases of non-cestode parasitic infections and malignancies (25). The third group of patients included subjects that did not react with *E. granulosus* antigens. IGF-I and IGF-II concentrations in patients with liver cysts were significantly lower than in healthy individuals of similar age and the fall was not sex-associated. The overall mean concentrations of IGF-I and IGF-II were similar whether the cysts were caused by *E. granulosus*, or cross-reactive pathologies, or arose from another origin.

Parasitic infections that affect the gastrointestinal or hepatobiliary tract induce metabolic changes that may vary depending on the parasite species, mode of acquirement, the way the parasite spreads in the host, its life cycle, expression, immunological response and the effects it causes. The response of the host to parasite invasion can produce more serious disease than the parasite itself. In addition to patients with echinococcosis, IGFs were examined in patients with trichinellosis and toxoplasmosis (26). It was found that infection with *Trichinella spiralis* led to a reduction in serum IGF-I concentration, while neither this parasite nor *Toxoplasma gondii* affected the level of IGF-II. In the subjects with trichinellosis, combined effects of inadequate nutrition and the immunological response probably occurred. Toxoplasmosis, although most often acquired via the gastrointestinal tract, did not seem to influence metabolic pathways involving IGF molecules.

IGFs and gastrointestinal inflammations

Gastrointestinal inflammations are immunologically mediated inflammatory diseases that are characterised by chronic inflammation with periods of clinical remission and reactivation (27). In most cases they include mucosal damage, epithelial cell destruction and ulceration, although in some cases they may develop towards tumors. Remission of symptoms in these patients includes repair and regeneration of damaged mucosa, so growth factors should be involved in mucosal regeneration. The patients included in our study had gastrointestinal inflammations of different etiology: Crohn's disease, colitis ulcerosa, gastritis, duodenitis errosiva, gastrointestinal candidiasis, rotaviral enteritis, adenoviral enteritis and several cases had inflammation of unknown cause (28).

The results showed that serum concentrations of IGFs in these patients were reduced compared to healthy subjects. Subgroups of patients were further analysed according to their primary diagnosis. IGF-I concentrations were significantly lower in all subgroups, whereas only patients with colitis ulcerosa, rotaviral and adenoviral enteritis and candidiasis had significantly decreased IGF-II (28, 29). A detailed statistical analysis demonstrated that the different stages

of gastrointestinal inflammation (categorised after endoscopy into stages 1, 2 and 3) showed no correlation with the changes in concentrations of the IGFs. Furthermore, it was not possible to establish any correlation between the intensity of the humoral immune response (i.e. serum antibody titer in the case of infection) and the concentrations of the IGFs. Serum cortisol concentration was significantly increased in the patients. No correlation between IGF-I or IGF-II concentration and the concentration of cortisol was found when the patients were analysed as one group. When the patients with the same etiology of the disorder were grouped separately, the IGF-I and cortisol concentrations exhibited a significant negative correlation in patients with Crohn's disease.

Gastritis is most often caused by spontaneous infection with the bacterium *Helicobacter pylori*. We investigated the IGF system in persons infected with *H. pylori* (diagnosed by endoscopy) and compared a group of patients having anti-*H. pylori* antibodies in their circulation with a group of individuals that were sero-negative (30). Both IGF-I and IGF-II concentrations were lower, and the concentration of cortisol higher than normal in these patients, regardless of the presence of specific antibodies in their circulation.

IGFs and gastric tumors

In a separate study, patients diagnosed with malignant gastric tumors were examined. All patients underwent open surgery and later suffered from post-operative sepsis. In 90% of the patients abdominal sepsis was diagnosed due to diffuse peritonitis caused by Gram positive cocci from the *Enterobacteriaceae* family, while urosepsis caused by *Escherichia coli* was diagnosed in the rest. The whole study was performed in order to obtain a complete picture of how the IGF system was affected successively by the three aforementioned conditions: primary disease, open surgery and sepsis.

The values for serum IGF-I and IGF-II in patients 24 h before surgery were significantly lower than those in healthy subjects. No difference between this stage and that 24 h after surgery was found, while post-operative sepsis induced a further reduction in IGF-I level. This decrease in serum IGF-I correlated with an increase in urinary IGF-I. Serum cortisol in patients before operation was significantly greater than in healthy subjects and sepsis led to an additional increase of cortisol in serum, as well as in urine. There was a negative correlation between serum cortisol and IGF-I. Glucose and insulin levels remained unchanged through all the stages of the study. Both serum albumin and total protein concentrations were significantly decreased in septic patients. However, the concentration of serum IgG did not alter, eliminating IgG, the second most abundant protein, as a participant in the alteration of total protein concentration.

IGFs and cholecystitis

The current view is that laparoscopy is a technique that is safer, less invasive and equally effective compared to traditional open surgery techniques. We performed a study on the influence of cholecystitis and laparoscopy on the IGF system (31). The results demonstrated that cholecystitis caused significant decrease in the concentrations of both IGF-I and IGF-II, but laparoscopy did not induce further changes. Two types of anaesthesia, balanced inhalatory (Sevoflurane) and intravenous (neuroleptic anaesthesia), administered to patients during laparoscopic cholecystectomy, were compared and contrasted. No difference between the two patient groups was found.

Analytical aspects of IGFBP determination

Native non-glycosylated IGFBP-3 has a molecular mass of 29 kD (32). There are three N-glycosylation sites (Asn-X-Ser/Thr) located at Asn^{89}, Asn^{109} and Asn^{172} (4, 4.5 and 5kD, respectively). In the circulation, IGFBP-3 exists as two glycoforms, the 40 kD glycoform with two occupied N-glycosylation sites and the 45 kD glycoform with the third variably glycosylated asparagine residue. IGFBP-3 can also be phosphorylated at Ser^{111} and Ser^{113}. While this has no effect on IGF-binding, binding to ALS is inhibited and phospho-IGFBP-3 is resistant to certain proteases (33).

Native IGFBP-1 has a molecular mass of 25 kD but is present in the normal circulation as a single highly phosphorylated isoform (34). While phosphorylation has no effect on IGF-II binding, it greatly increases the affinity of IGFBP-1 for IGF-I to a level significantly higher than that of IGFBP-3, the large normally saturated IGF reservoir. IGFBP-1 may function independently of the IGFs through its RGD sequence by inhibiting fibronectin binding to alpha5beta1 integrin. It has been identified as a possible hepatic survival factor by suppression of apoptotic and necrotic pathways induced during acute liver injury (35).

Thus, a variety of pathological conditions characterised by increased catabolism may affect the IGFBP-3 and IGFBP-1 in the circulation. As stated before, the roles of other IGFBPs in the circulation are less well understood. In our studies, we investigated the influence of the above-mentioned pathologies on the concentration and structure of some IGFBPs.

Large prospective studies have identified high levels of serum IGF-I and low levels of serum IGFBP-3 as risk factors in the development of prostate, colorectal and breast cancer. However, other studies considering breast and prostate cancer have given conflicting results, i.e. the level of IGFBP-3 is decreased, increased or unchanged (36). Some of these contradictions probably result from different techniques for IGFBP-3 measurement. Some commercial kits determine total IGFBP-3 (including fragments), whereas

others measure intact (IGF-binding) IGFBP-3. In order to validate the exactness and usefulness of the information about the serum concentration of IGFBP-3, we analysed several aspects of IGFBP-3 determination (37). Since alterations in IGFBP-3 concentration may also be accompanied by changes in its carbohydrate content, the presence of certain sugars in the carbohydrate moiety of IGFBP-3 was examined, using lectin-affinity chromatography, lectin electrophoresis and a solid-phase lectin-binding assay. The glycosylation pattern may affect the susceptibility of IGFBP-3 to proteolysis and, subsequently, its amount and function.

The IGFBP-3 concentration was measured by an immunoradiometric assay (IRMA-IGFBP-3, INEP, Belgrade, Serbia) (37). An overview of the results for IGFBP-3 obtained in specific groups of patients is presented in *Table I*. As for IGF-I and IGF-II, numerical data are expressed as the mean value and SD. Differences between the groups were analysed by the non-parametric Mann-Whitney U test. An immunoradiometric assay for the determination of IGFBP-1 (IRMA-IGFBP-1, INEP, Belgrade, Serbia) has just been formulated and investigations on IGFBP-1 concentrations in patients' samples are currently being conducted.

IGF-binding proteins in serum were also analysed by ligand and immunoblotting, after SDS electrophoresis and transfer to nitrocellulose membranes (28). Immunoblotting offers information on the degree of IGFBP proteolysis, type of fragmentation and the relative ratio of specific fragments to intact molecules. In addition, the relative ratio of the two characteristic intact IGFBP-3 isoforms can be evaluated. Occasionally, a peculiar molecular species (modified by disease) may be found. In one such serum (from a patient with a gastric tumor treated by surgery, who then acquired sepsis) we detected an anti-IGFBP-3 antibody reactive protein having molecular mass greater than 45 kD, suggesting structural, and possibly functional, transformation of the innate IGFBP-3 protein to an abnormal species due to illness (37).

IGFBPs in hepatobiliary and gastrointestinal diseases

In patients with liver cirrhosis, liver cysts, gastrointestinal inflammation (caused by *H. pylori* infection or due to other causes), cholecystitis and gastric cancer, the amount of IGFBP-3 was found to decrease and the amounts of IGFBP-2 and IGFBP-1 to increase in the circulation (22, 24, 28–31). In cirrhosis the alteration was more pronounced with progression of the disease from stage A to C (22). In patients with gastrointestinal inflammation the amount of proteolysed IGFBP-3 fragments was similar to that in healthy individuals (28), while in patients with gastric cancer the relative amount of fragments increased, as also occurred in patients with sepsis. Increased amounts of serum IGFBP-1, IGFBP-2 and IGFBP-4 were measured in patients with trichinellosis (26). No change in the levels of IGFBP-1, IGFBP-2 and IGFBP-3 due to intervention was observed in patients undergoing laparoscopic cholecystectomy or open surgery (patients with gastric cancer), but sepsis induced further reduction of IGFBP-3, while IGFBP-1, IGFBP-2 and IGFBP-4 increased several fold (31, 38).

In comparison with healthy individuals, the IGFBP-3 in patients with liver cirrhosis exhibited diminished interaction with concanavalin A, wheat-germ agglutinin and breadfruit lectin, suggesting structural changes in oligo-Man, GlcNAc and Gal carbohydrate units (39). Up to twelve charged species of IGFBP-3 have been identified (40). However, in patients with liver cysts IGFBP-3 demonstrated similar reactivity towards lectins as IGFBP-3 from healthy persons (24), suggesting that the overall structure of the saccharide moieties of IGFBP-3 was not significantly altered due to cyst formation and that physiological interactions dependent on carbohydrate recognition are, most likely, unaffected. The saccharide structure of IGFBP-3 was also conserved in patients with gastrointestinal inflammation (28).

A significant increase in the sialic acid content of IGFBP-3 was seen in patients with cholecystitis (38). Using *Sambucus nigra* agglutinin (SNA) affinity chromatography, we found increased binding of IGFBP-3 to SNA, a plant lectin specific for NeuA$\alpha\rightarrow$6Gal sequences. This increase in the content of terminal sialic acid may have been a consequence of increased activity of sialyltransferases. Increased sialyltransferase activity has been observed in the sera of patients with brain and thyroid cancer, as well as in patients with acute myeloid leukemia or multiple myeloma (41). A higher degree of sialylation may not be due only to increased sialyltransferase activity, but also to an increase in N-glycan branching which provides more substrate for sialylation. Furthermore, the activity of some sialidases may have been decreased. Increased sialylation of IGFBP-3 may serve to protect IGFBP-3 from rapid degradation, to prolong its half-life in the circulation and to maintain its function.

As for the IGF-I : IGFBP-3 molar ratio, it was calculated to be 0.26 ± 0.071 in the reference (healthy) group of individuals, 0.21 ± 0.057 in patients with gastrointestinal inflammation, 0.13 ± 0.041 in patients with gastric tumors, 0.12 ± 0.044 in patients with postoperative sepsis and 0.15 ± 0.056 in patients with cholecystitis. The relative amount of IGF-I obviously decreased to a greater extent than the relative amount of IGFBP-3, much of which may be occupied with IGF-II, pointing to IGF-I as the most sensitive biomarker of catabolism within the IGF-IGFBP system in the circulation.

Conclusion

There is ample evidence that changes in the proteins of the IGF-IGFBP axis play an important role in the metabolic disturbances associated with illness. It remains to be determined whether the measurement of these proteins will provide diagnostic or prognostic tests of value in the management of patients suffering from severe catabolism.

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References

1. Mauras N, Haymond MW. Are the metabolic effects of GH and IGF-I separable? Growth Horm IGF Res 2005; 15: 19–27.
2. O'Dell SD, Day IN. Insulin-like growth factor II (IGF-II). Int J Biochem Cell Biol 1998; 30: 767–71.
3. Kim JJ, Accilli D. Signaling through IGF-I and insulin receptors: where is the specificity? Growth Horm IGF Res 2002; 12: 84–90.
4. Kupfer SR, Underwood LE, Baxter RC, Clemmons DR. Enhancement of the anabolic effects of growth hormone and insulin-like growth factor I by use of both agents simultaneously. J Clin Invest 1993; 91: 391–6.
5. Rinderknecht E, Humbel RE. The amino acid sequence of human IGF-I and its structural homology with proinsulin. J Biol Chem 1978; 253: 2769–76.
6. Collett-Solberg PF, Cohen P. Genetics, chemistry and function of the IGF/IGFBP system. Endocrine 2000; 12: 121–36.
7. Butler AA, Le Roith D. Control of growth by somatotropic axis: growth hormone and the insulin-like growth factors have related and independent roles. Ann Rev Physiol 2001; 63: 141–64.
8. Volkl TMK, Schwobel K, Simm D, Beier C, Rohrer TR, Dorr HG. Spontaneous growth hormone secretion and IGF-I : IGFBP-3 molar ratios in children born small for gestational age (SGA). Growth Horm IGF Res 2004; 14: 455–61.
9. Baxter RC. Changes in the IGF – IGFBP axis in critical illness. Best Pract Res Clin Endocrinol Metab 2001; 15: 421–34.
10. Hwang DL, Huang SP, Lan WS, Lee PDK. Elevated insulin, proinsulin and insulin-like growth factor-binding protein-1 in liver disease. Growth Horm IGF Res 2003; 13: 316–21.
11. Nygren J, Sammann M, Malm M, Efendic S, Hall K, Brismar K, et al. Distributed anabolic hormonal patterns in burned patients: the relation to glucagon. Clin Endocrinol 1995; 43: 491–500.
12. Baxter RC, Hawker FH, To C, Stewart PM, Holman SR. Thirty-day monitoring of insulin-like growth factors and their binding proteins in intensive care unit patients. Growth Horm IGF Res 1998; 8: 455–63.
13. Juul A. Serum levels of insulin-like growth factor I and its binding proteins in health and disease. Growth Horm IGF Res 2003; 13: 113–70.
14. Nikolić JA, Nedić O, Masnikosa R. Peripheral serum concentrations of insulin-like growth factors (IGF-I and IGF-II) decrease with age in healthy adults. Genetika 2000; 32: 155–65.
15. Nikolić JA, Nedić O, Ratković M, Masnikosa R. Alteration in serum insulin-like growth factor (IGF-I) levels during the menstrual cycle. Jugoslov Med Biohem 1998; 17: 15–20.
16. Moyano P, Rotwein P. Mini-review: estrogen action in the uterus and insulin-like growth factor-I. Growth Horm IGF Res 2004; 14: 431–5.
17. Gargosky SE, Hasegawa T, Tapanainen P, MacGillivray M, Hasegawa Y, Rosenfeld R. Urinary insulin-like growth factors (IGF) and IGF-binding proteins in normal subjects, growth hormone deficiency and renal disease. J Clin Endocrinol Metab 1993; 76: 1631–7.
18. Nikolić JA, Masnikosa R. Determination of insulin-like growth factors in bovine milk and colostrum by radioimmunoassay. Acta Vet Beograd 1998; 48: 115–23.
19. Nikolić JA, Nedić O, Mihajlović N, Baričević I. Insulin-like growth factor-I and -II and their binding proteins in human ejaculates. Jugoslov Med Biohem 2003; 22: 221–7.
20. Nikolić JA, Ratković M, Nedić O. Determination of insulin-like growth factor-I by radioimmunoassay. J Serb Chem Soc 1996; 61: 1149–57.
21. Nikolić JA, Nedić O, Masnikosa R. Determination of insulin-like growth factor-II in human serum. J Serb Chem Soc 1998 , 63: 805–15.
22. Nikolić JA, Todorović V, Božić M, Tošić Lj, Bulajić M, Alempijević T, et al. Serum insulin-like growth factor (IGF-II) is more closely associated with liver dysfunction than is IGF-I in patients with liver cirrhosis. Clin Chim Acta 2000; 294: 169–77.
23. Mortle KJ, Ros PR. Cystic focal liver lesions in the adult: differential CT and MR imaging features. Radiographics 2001; 21: 895–910.
24. Nedić O, Nikolić JA, Baričević I, Jovanović B, Ilić N. Insulin-like growth factors in patients with liver cysts. J Clin Lab Anal 2004; 18: 299–304.
25. Poretti D, Felleisen E, Grimm F, Phister M, Teuscher F, Zuercher C, et al. Differential immunodiagnosis between cystic hydatid disease and other cross-reactive pathologies. Am J Trop Med Hyg 1999; 60: 193–8.
26. Nedić O, Nikolić JA, Baričević I, Jovanović B, Ilić N. Insulin-like growth factors and their binding proteins in the circulation of patients with echinococcosis, trichinel-

losis and toxoplasmosis. Clin Chim Acta 2003; 335: 83–8.

27. Katsanos KH, Tsatsoulis A, Christodoulou D, Challa A, Katsaraki A, Tsianos EV. Reduced serum insulin-like growth factor-I (IGF-I) and IGF-binding protein-3 levels in adults with inflammatory bowel disease. Growth Horm IGF Res 2001; 11: 364–7.
28. Baričević I, Jones D, Nikolić JA, Nedić O. Gastrointestinal inflammation and the circulating IGF system in humans. Horm Metab Res 2006; 38: 22–7.
29. Baričević I, Nedić O, Nikolić JA, Nedeljković J. The insulin-like growth factor system in the circulation of patients with viral infections. Clin Chem Lab Med 2004; 42: 1127–31.
30. Baričević I, Nedić O, Nikolić JA, Bojić B, Jojić Nj. Circulating insulin-like growth factors in patients infected with Helicobacter pylori. Clin Biochem 2004; 37: 997– 1001.
31. Baričević I, Jones D, Malenković V, Nedić O. The effect of laparoscopy on the IGF system in patients diagnosed with acute cholecystitis. Clin Endocrinol 2006; 65: 373–9.
32. Firth SM, Baxter RC. Characterisation of recombinant glycosylation variants of insulin-like growth factor binding protein-3. J Endocrinol 1999; 160: 379–87.
33. Coverley JA, Martin JL, Baxter RC. The effect of phosphorylation by casein kinase 2 on the activity of insulin-like growth factor-binding protein-3. Endocrinol 2000; 141: 564–70.
34. Westwood M, Gibson JM, White A. Purification and characterization of the insulin-like growth factor-binding protein-1 phosphoform found in normal plasma. Endocrinol 1997; 138: 1130–6.
35. Leu JI, Crissey MAS, Taub. Massive hepatic apoptosis associated with TGF-β1 activation after Fas ligand treatment of IGF binding protein-1-deficient mice. J Clin Invest 2003; 111: 129–39.
36. Wallace P. Can [IGF-I] & [IGFBP-3] be diagnostic for cancer? Technical Bulletin GroPep Limited 2004; 10.
37. Nedić O, Jones D, Golubović S, Baričević I. New considerations for IGFBP-3 determination in human serum. Clin Chim Acta 2005; 337: 211–3.
38. Baričević I, Malenković V, Jones DR, Nedić O. The influence of laparoscopic and open surgery on the concentration and structural modifications of insulin-like growth factor binding protein 3 in the human circulation. Acta Physiol Hung 2006; 93: 361–9.
39. Nedić O, Nikolić JA, Hajduković-Dragojlović Lj, Todorović V, Masnikosa R. Alterations of IGF-binding proteins in patients with alcoholic liver cirrhosis. Alcohol 2000; 21: 223–9.
40. Nedić O, Nikolić JA, Prišić S, Aćimović J, Hajduković-Dragojlović Lj. Reactivity of IGF binding protein-3 isoforms towards concanavalin A in healthy adults and subjects with liver cirrhosis. Addict Biol 2003; 8: 81–8.
41. Brockhausen I, Kuhns W. Glycoproteins and human disease. Heidelberg: Springer Verlag, 1997.

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