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ScienceDirect
Metabolism Clinical and Experimental 57 (2008) 658–661
Metabolism
Clinical and Experimental
www.metabolismjournal.com

The change in the circulating insulin-like growth factor binding protein 1 isoform pattern during the course of oral glucose tolerance test

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Received 19 September 2007; accepted 6 December 2007

Abstract

There is a tight connection between insulin-like growth factor binding protein 1 (IGFBP-1) and nutrient/energy supply, suggesting modulation of the short-term insulin-like activity and glucose homeostasis by IGFBP-1. Differential phosphorylation of IGFBP-1 alters its affinity for insulin-like growth factors (IGFs) and its capacity to modulate cellular response. The object of this study was to define the time course of changes in the IGFBP-1 isoform pattern during an oral glucose tolerance test. Besides changing in counterdirections, the alterations in glucose/insulin/C-peptide and IGF-I/IGFBP-1 concentrations were phase-shifted. Denaturing electrophoresis revealed that the IGFBP-1 proteolytic activity was not increased after glucose ingestion. In native electrophoresis, the isoform that moved most anodically, with the greatest phosphate content, was markedly reduced during the course of oral glucose tolerance test; and it disappeared after 3 hours. Our data show that both a change in the total amount of IGFBP-1 and an alteration in the relative amount (ratio) of the specific phosphoforms of IGFBP-1 are part of the mechanism involved in modulation of the insulin-like activity.

1. Introduction

Insulin-like growth factor binding protein 1 (IGFBP-1) is a 25-kd protein, an important regulator of the bioavailability of the insulin-like growth factors (IGF-I and IGF-II), peptides that have a key role in the metabolism, development, and growth of many tissues and organs [1]. Insulin-like growth factor binding protein 1 is synthesized in a variety of cell types, including hepatocytes, ovarian granulose cells, and decidualized endometrium. In healthy, nonpregnant individuals, circulating IGFBP-1 is mostly derived from the liver. Insulin-like growth factor binding protein 1 is transcriptionally repressed by insulin, and its concentration in blood fluctuates daily in response to changes in insulin concentration. It has been associated with insulin sensitivity, metabolic syndrome, cardiovascular risk factors, and possibly cancer [2].

Insulin-like growth factor binding protein 1 was reported to both inhibit and stimulate the actions of IGFs. These apparently opposing observations may be explained by the recent finding that differential phosphorylation of IGFBP-1 could significantly alter its affinity for IGFs and, therefore, its capacity to modulate the cellular response [3]. It has been demonstrated that nonphosphorylated IGFBP-1 (npIGFBP-1) has 4- to 6-fold lower affinity for IGF-I compared with phosphorylated variants (pIGFBP-1). The potential phosphorylation sites are serine residues 101, 119, and 169; and as many as 5 IGFBP-1 variants differing in their degree of phosphorylation have been reported [4].

The predominant form found in the circulation of healthy adults is a highly phosphorylated IGFBP-1 [5]. As IGFBP-1 regulates the bioavailability of free IGFs and, thus, the insulin-like activity, it may indirectly control the glucose-lowering potential in the organism in relation to nutrient/energy supply. A degree of IGFBP-1 phosphorylation may have further impact on the maintenance of glucose homeostasis and the insulin/IGFBP-1 axis coordination. Overexpression of the IGFBP-1 leads to impaired glucose tolerance and abnormalities of insulin action [6]. The object of our study was to define the time course of changes in the IGFBP-1 isoform pattern in healthy subjects undergoing an oral glucose tolerance test (OGTT).

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doi:10.1016/j.metabol.2007.12.010

2. Materials and methods

2.1. Blood samples

Healthy adult volunteers (n = 28; 12 men and 16 women; age, 27-56 years; body mass index, 20-31 kg/m^2) were subjected to OGTT (75 g of glucose dissolved in 0.2 L of water) after an overnight (12 hours) fast. Venous blood samples were collected immediately before OGTT (0 hour) and at 1-hour time intervals during the next 3 hours (1, 2, and 3 hours). Each time, the serum was separated by centrifugation within 45 minutes. Samples were assayed immediately for glucose and within a week for IGF-I, IGFBP-1, insulin, and C-peptide. Meanwhile, sera were kept frozen at −20°C. An OGTT was done on the volunteers to define potential changes in the IGFBP-1 isoform pattern. The study was approved by the local ethics committee of the Institute for the Application of Nuclear Energy, and all subjects gave informed consent to participate.

2.2. Measurement of serum parameters

Glucose concentration was measured using RANDOX (Crumlin, United Kingdom) reagent (glucose oxidase–peroxidose [GOD-PAP] method); the reference levels according to the producer are 4.2 to 6.4 mmol/L. The IGF-I, insulin, and C-peptide levels were determined by the Institute for the Application of Nuclear Energy (Belgrade, Serbia) commercial radioimmunoassay kits; the reference values are 15 to 40 nmol/L for IGF-I, 5 to 25 mU/L for insulin, and 0.3 to 0.7 nmol/L for C-peptide. The IGFBP-1 concentration was measured by DSL (Webster, TX) Total IGFBP-1 IRMA kit, the reference limits being 0.04 to 1.80 nmol/L. Numerical data were presented as means (x) and standard deviation (SD) and statistically analyzed using the Student t test for the significance of differences between the time intervals.

2.3. Polyacrylamide gel electrophoresis and immunoblotting

All serum samples were subjected to both denaturing (sodium dodecyl sulfate–polyacrylamide gel electrophoresis [SDS-PAGE]) and native (nPAGE, without detergent) electrophoresis [7], followed by immunoblotting using goat polyclonal anti–IGFBP-1 antibody (DSL) [8]. The membranes were further incubated with an anti–goat immunoglobulin G antibody coupled to horseradish peroxidase (Biosource, Camarillo, CA), and immunoreactive proteins were visualized with an enhanced chemiluminescence kit (Amersham, Aylesbury, United Kingdom). The SDS-PAGE served to detect molecular species according to their mass (native and fragmented IGFBP-1), whereas nPAGE was used to separate differently charged IGFBP-1 molecules. The relative presence of each IGFBP-1 isoform was estimated from the immunoblots obtained after nPAGE using densitometric analysis of the protein bands. Densitometric evaluation was performed with ImageMaster TotalLab Software (Amersham Biosciences, Newcastle, United Kingdom).

3. Results

Numerical data for the measured parameters are shown in Table 1. Individuals included in this study exhibited the expected changes in the concentration of glucose, insulin, and C-peptide during OGTT, confirming that the group consisted of healthy subjects in respect to glucose regulation [9]. The basal levels of IGF-I and IGFBP-1 fell within the reference ranges, suggesting normal hepatic synthesis of these proteins.

The concentrations of glucose, insulin, and C-peptide were the greatest 1 hour after glucose intake and then decreased, approaching fasting level after 2 hours (glucose) and 3 hours (insulin), respectively. The IGF-I concentration did not change significantly during OGTT, although it was lower after the OGTT had started compared with the initial sample and continued to fall during the examined period. The IGFBP-1 concentration was significantly reduced 2 hours after the OGTT had started and continued further to drop at 3 hours. Besides changing in counter-directions, the alterations in glucose/insulin/C-peptide and IGF-I/IGFBP-1 concentrations were phase-shifted. This shift resulted from a time-delayed reaction within insulin/IGFBP-1 axis, and it adjusted (decreased) the level of IGFBP-1 after the primary event—the elevation of insulin.

All sera were subjected to both SDS-PAGE and nPAGE, but only representative samples are shown in Fig. 1 (sera from all patients displayed the same general pattern of IGFBP-1 alteration). The aim of the SDS-PAGE was to determine the possible existence of proteolytic IGFBP-1 fragments. Autoradiogram obtained after SDS-PAGE/immunoblotting and long exposition time (30 minutes) is shown in Fig. 1A. Twelve samples from 3 persons (A, B, and C) subjected to OGTT, represented by 4 specimens at the time

Table 1
The concentrations of glucose, IGF-I, IGFBP-1, insulin, and C-peptide in healthy subjects during OGTT

Time interval (h)	Glucose (mmol/L) $x \pm$ SD	IGF-I (nmol/L) $x \pm$ SD	IGFBP-1 (nmol/L) $x \pm$ SD	Insulin (mU/L) $x \pm$ SD	C-peptide (nmol/L) $x \pm$ SD
0	5.1 ± 0.64	17.3 ± 3.73	0.7 ± 0.46	15 ± 4.4	0.5 ± 0.17
1	8.4 ± 0.94**	16.9 ± 4.47	0.7 ± 0.47	110 ± 51.3**	2.0 ± 0.64**
2	5.5 ± 0.80**	16.5 ± 4.06	0.4 ± 0.27*	42 ± 24.5**	1.7 ± 0.58*
3	4.9 ± 0.71	16.2 ± 4.23	0.2 ± 0.16*	16 ± 4.9*	1.4 ± 0.48*

Results are presented as means (x) and SD. Statistically significant differences between successive time intervals are indicated as *$P < .05$ and **$P < .0005$, respectively.

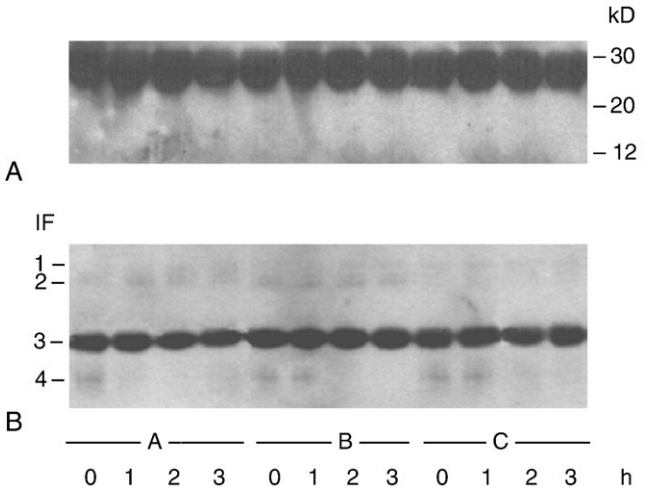

Fig. 1. The SDS-PAGE (A) and nPAGE (B) followed by immunoblotting of serum samples from 3 persons subjected to OGTT (A, B, and C), represented by 4 specimens at time intervals 0, 1, 2, and 3 hours each. The IGFBP-1 isoforms (IF) are indicated on the left-hand side, and the molecular mass markers are on the right.

intervals 0, 1, 2, and 3 hours each, demonstrated that there was a very intensive protein band between 20 and 30 kd and that there were no visible IGFBP-1 fragments.

In nPAGE, at least 4 IGFBP-1 isoforms separated one from another, 1 predominant and 3 minor. As electrophoretic migration toward anode increases with negative charge density, IGFBP-1 isoforms with greater phosphate content moved faster. The same samples represented in Fig. 1A are shown in Fig. 1B, but after nPAGE. The relative intensities of the protein bands corresponding to isoforms 1, 2, and 3 (IF1, IF2, and IF3) changed less than the intensity of the fourth isoform (IF4), which was markedly reduced during the course of OGTT. Two hours after glucose administration, the protein band corresponding to IF4 almost disappeared; and after 3 hours, it was no longer visible under experimental conditions applied. The abundance of IGFBP-1 isoforms was estimated by densitometric analysis of protein bands. The relative amounts ranged from 1.0% to 1.8% for isoforms IF1 + IF2, 85.4% to 91.3% for IF3, and 6.4% to 9.9% for IF4 in the sera collected before OGTT. In the samples obtained 2 hours after OGTT started, the IF4 protein band was almost undetectable in most cases, whereas at the time interval of 3 hours, IF4 could not be distinguished from the background.

4. Discussion

Phosphorylation is a very important posttranslational modification of proteins that significantly alters their physical characteristics, such as charge and conformation, and biochemical characteristics that include interactions with other molecules [10]. Ligand-binding affinity, proteolytic stability, and nearly entire cell-signaling pathways depend on the state of protein phosphorylation.

Changes in the phosphorylation of IGFBP-1 influence the bioavailability of IGFs and cause significant alteration of its immunoreactivity. Quantitative determination of IGFBP-1 in various biological samples represented a serious problem until recently, as the results obtained with different immunoassays were several-fold different [1,11]. The variations were not even of the same magnitude for various sample types. All disagreements were finally attributed to specific immunoreactivity of the phosphorylated and nonphosphorylated isoforms. In this study, we used a commercial immunoassay—DSL Total IGFBP-1 IRMA—that is, according to the producer's manual, unaffected by the state of IGFBP-1 phosphorylation [12]. For the same reason, we used polyclonal anti–IGFBP-1 antibody in immunoblotting, expecting to detect IGFBP-1 isoforms regardless of their phosphorylation status.

The SDS-PAGE revealed that the IGFBP-1 proteolytic activity was not increased after glucose ingestion, and there were no visible IGFBP-1 fragments upon autoradiography. Human serum has been shown to contain proteases that degrade different IGFBPs; but, to date, no such protease has been identified for IGFBP-1. Gibson et al [13] investigated the stability of IGFBP-1 and found that npIGFBP-1 was cleaved by both decidual cell proteases, namely, placental alkaline phosphatase and plasmin, whereas pIGFBP-1 was resistant to proteolysis. The same group of authors reported association between pIGFBP-1 and α_2-macroglobulin in human serum [4]. They concluded that α_2-macroglobulin protects IGFBP-1, which can still bind IGFs, from proteolysis.

The absence of IGFBP-1 fragments in our serum samples after OGTT permitted IGFBP-1 isoform analysis in nPAGE. Native PAGE revealed reduction in the amount of the IGFBP-1 isoform that moved most anodically. Iwashita et al [3] demonstrated that npIGFBP-1 was the molecular form that remained the closest to cathode in nondenaturing PAGE and that all phosphorylated isoforms, after treatment with alkaline phosphatase, also migrated as one protein band corresponding to npIGFBP-1. According to our results, the minor pIGFBP-1 isoform that was the most phosphorylated (IF4) decreased the most during the course of OGTT. Because pIGFBP-1 has a several-fold higher affinity for IGF-I compared with npIGFBP-1, combination of the relative abundance of IF4 (up to 10% of the total amount of IGFBP-1) and its affinity for IGF-I may significantly influence the bioavailability of IGF-I. The disappearance of the IGFBP-1 isoform with the greatest affinity for IGF-I causes the redistribution of the ligand among other IGFBP-1 isoforms and, possibly, other IGFBPs. This redistribution leads to an increase in IGF-I bioavailability, which exerts insulin-like metabolic functions [14].

According to our results, total IGF-I concentration decreased slightly, but not significantly, during OGTT. Similar finding was reported by Frystyk et al [15], but they also reported significant decrease in the concentration of free IGF-I after 4-hour OGTT and an inverse correlation between

free IGF-I and IGFBP-1 in the late postprandial phase. Chen et al [16], as well, determined unchanged total IGF-I, whereas bioactive IGF-I declined significantly, after an oral glucose load.

Although IGFBP-1 binds only a minor portion of the circulating IGFs, it has received considerable attention because of its unique properties. The tight connection between IGFBP-1 and nutrient/fuel supply suggests modulation of the short-term insulin-like activity and glucose homeostasis by IGFBP-1. Close relation between IGFBP-1 and glucose metabolism was confirmed by several independent experiments demonstrating that IGFBP-1 was increased in vivo by glucose counterregulatory hormones such as glucagon, catecholamines, and glucocorticoids [17]; that increase in serum IGFBP-1 during exercise was related to the decrease in liver glycogen content [18]; that intake of carbohydrates (or more specifically, starch) was positively correlated with IGFBP-1 levels, possibly due to induced hepatic insulin resistance [2]; that IGFBP-1 decreases with obesity [19]; and that ghrelin positively correlates with IGFBP-1 [20].

Brismar and colleagues [21] found no significant reduction in hepatic IGFBP-1 secretion within the first hour of insulin infusion in humans, although there was a tendency for blood IGFBP-1 concentration to fall. A significant decline in the hepatic production of IGFBP-1 was observed afterward, and hepatic output was completely blocked after 2 hours. The mean estimated half-life of peripheral venous IGFBP-1 was between 1.5 and 2 hours. There are 2 proposed mechanisms of the effect of insulin: within the first hour, insulin increases the clearance of IGFBP-1 from the circulation; and later, insulin inhibits hepatic IGFBP-1 gene transcription. The role of IGFBP-1 in the regulation of insulin is also poorly understood. Zhang et al [22] found attenuation of insulin secretion by IGFBP-1 in isolated pancreatic β-cells and, in contrast, augmentation of insulin secretion in the intact islets. We have shown that, besides the change in the total amount of IGFBP-1, the modulation of the insulin-like activity is achieved by the alteration in the relative amount (ratio) of the specific phosphoforms, suggesting different clearing rates of IGFBP-1 isoforms, as part of the mechanism involved in glucose homeostasis.

Acknowledgment

This work was supported by the Ministry of Science of Serbia grant 143019.

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