Available online at www.sciencedirect.com
ScienceDirect
Clinical Biochemistry 42 (2009) 1270–1274
CLINICAL BIOCHEMISTRY

Biological variation in fasting serum insulin-like growth factor binding protein-1 (IGFBP-1) among individuals with a varying glucose tolerance

Anwar Borai [a,*], Callum Livingstone [a,b], Shweta Mehta [b], Hawazen Zarif [c], Fatima Abdelaal [c], Gordon Ferns [a,b]

[a] *Faculty of Health and Medical Sciences, University of Surrey, Guildford, GU2 7XH, Guildford, UK*
[b] *Department of Clinical Biochemistry, Royal Surrey County Hospital, Guildford, UK*
[c] *Department of Medicine, King Khalid National Guard Hospital, Jeddah, Saudi Arabia*

Received 3 March 2009; received in revised form 26 April 2009; accepted 4 May 2009
Available online 20 May 2009

Abstract

Objective: To evaluate the biological variation of serum insulin-like growth factor binding protein-1 (IGFBP-1) in individuals with different degrees of glucose tolerance.

Research design and methods: IGFBP-1 was measured in 42 fasted subjects with: normal glucose tolerance (NGT=15), impaired fasting glucose (IFG=9), impaired glucose tolerance (IGT=9) and type 2 diabetics (DM=9). Short- and long-term reproducibility was determined for IGFBP-1.

Results: The longer-term reproducibility (CV) of serum IGFBP-1 was 20.9%, 29.5%, 33.1% and 48.0% for subjects with NGT, IFG, IGT and DM respectively. The results for the short-term serum IGFBP-1 yielded CVs for these same respective categories of 8.2%, 16.1%, 29.2% and 13.8%.

Conclusions: IGFBP-1 measurement was least variable in NGT individuals over both long-term and short-term intervals and variability increased with deteriorating glucose tolerance. Due to its biological variation, IGFBP-1 serum level is more reliable in normal subjects than in individuals with type 2 diabetes.

Keywords: IGFBP-1; FPI; Reproducibility; Biological variation

Introduction

Insulin-like growth factor binding protein-1 (IGFBP-1) is a plasma protein whose concentrations are inversely related to fasting plasma insulin (FPI). Its concentration also appears to be a good correlate of insulin resistance as estimated by a hyperinsulinaemic euglycaemic clamp in normal subjects ($r=0.73$, $p<0.004$) [1]. Furthermore, we have recently shown [2] that insulin sensitivity (Si) derived from the frequently sampled intravenous glucose tolerance test (FSIVGTT) is more strongly correlated to serum IGFBP-1 ($r=0.79$, $p<0.0001$) than to several other simple measures of insulin sensitivity in normal subjects. The measurement of IGFBP-1 has other potential clinical applications in addition to the assessment of insulin sensitivity [3]. As an early marker of insulin resistance however, IGFBP-1 needs further validation including data on intra-individual biological variation, which has not been reported previously. With a similar rationale, Jaygobal et al. [4] have previously investigated the biological variation of sex hormone binding globulin (SHBG). We have therefore assessed the reproducibility of serum IGFBP-1 and FPI over long- and short-term intervals, and have also investigated the biological variation in individuals with different degrees of glucose tolerance.

Subjects

Reproducibility of IGFBP-1 and FPI over the long-term interval was determined in subjects from different glycaemic categories over two visits separated by an average of 10 days. All subjects gave informed consent for participation and the study was approved by the South West Surrey Local Research Ethics Committee, Guildford, UK. The forty two subjects were classified into subcategories without significant differences in

* Corresponding author. Fax: +44 1483 464072.
E-mail address: a.al-borai@surrey.ac.uk (A. Borai).

0009-9120/$ - see front matter © 2009 The Canadian Society of Clinical Chemists. Published by Elsevier Inc. All rights reserved.
doi:10.1016/j.clinbiochem.2009.05.005

Table 1
Clinical and biochemical characteristics for subjects in different glycaemic categories.

	NGT (n=15)	IFG (n=9)	IGT (n=9)	DM (n=9)
Male (%)	60%	67%	77%	62%
Age (years)	41±4	48±2	53±3	48±4
BMI (kg/m^2)	24.6±1.2	30.5±1.0	30.4±1.1	31.8±1.4
HbA1c (%)	5.4±0.1	6.0±0.2	5.8±0.2	7.2±0.5
FPG (1st)	5.3±0.1	6.3±0.1	6.5±0.2	8.2±0.9
FPG (2nd)	5.2±0.1	6.0±0.1	6.3±0.2	7.4±0.9
FPI (1st)	68±7	108±17	134±18	130±20
FPI (2nd)	57±6	89±12	94±16	86±15
IGFBP-1 (1st)	6.1±1.4	3.0±0.7	2.2±0.6	8.8±1.5
IGFBP-1 (2nd)	6.3±1.8	2.2±0.6	1.7±0.4	4.6±1.3

Fasting glucose, insulin and IGFBP-1 results are included in visit 1 (1st) and visit 2 (2nd).
Data are mean±SEM. Key: BMI, body mass index; HbA1c, glycated haemoglobin; FPG, fasting plasma glucose; FPI, fasting plasma insulin; NGT, normal glucose tolerance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; DM, type 2 diabetes.

gender as shown in Table 1. A standard 75 g oral glucose tolerance test (GTT) was carried out at the first visit and subjects were classified according to WHO criteria [5] as normal glucose tolerance NGT (n=15), impaired fasting glucose IFG (n=9), impaired glucose tolerance IGT (n=9) and type 2 diabetes (n=9). Normal subjects were recruited from hospital staff while other subjects were recruited from the lipid clinic of Royal Surrey County Hospital. Subjects with type 2 diabetes were carefully selected who had been managed by dietary means alone and not previously received oral antidiabetic medications (e.g. metformin) or insulin. None of the subjects were being treated with steroids or thyroxine which could modify insulin levels. All subjects were asked to have an unrestricted diet and not to modify their diet or levels of physical activity before visits 1 and 2. The subjects were advised to refrain from excessive exercise, smoking and alcohol before each visit. Glycated haemoglobin (HbA1c) levels were requested to check their glycemic control. In order to investigate reproducibility over the short-term, FPI and IGFBP-1 were measured four times in the fasting state at 5 min intervals during the second visit.

Methods and materials

Insulin was measured by solid phase two-site ELISA (Mercodia, Uppsala, Sweden) which had a sensitivity of 7.0 pmol/L and maximum analytical CV of 4.9%. Glucose was assayed on Bayer Advia 1650 auto-analyser using the hexokinase enzyme principle. Glycated haemoglobin (HbA1c) analysis was by a high pressure liquid chromatography technique on a Biorad Variant II instrument.

IGFBP-1 electrophoresis and immunoblotting

In order to validate the R&D IGFBP-1 antibody (cat. no. MAB675), serum samples were analyzed by immunoprecipitation, alkaline phosphatase dephosphorylation, electrophoresis and Western ligand blotting. For immunoprecipitation a Roche Protein G kit (cat no. 94001) was used. Briefly 1 μg of antiphosphoserine antibody (BD cat no. 612547) was added to 1 mL of serum sample and gently rocked for 1 h. 50 μL of protein G suspension beads was then added to the mixture and incubated overnight on a rocking platform at 2–8 °C. After centrifugation at 12,000 ×g for 20 s the supernatant was discarded. Wash buffer (1 mL) was then added to the beads and incubated for 20 min on a rocking platform. After repeating the washing procedure three times the last trace following the final wash was used for electrophoresis. Dephosphorylation technique of IGFBP-1 was achieved by treating 200 μL of serum sample with 200 U of calf serum alkaline phosphatase (Roche, cat. no. 38001) then dissolved in 10 μL of 1 mol/L diethanolamine containing 0.5 mmol/L $MgCl_2$. The mixture incubated at room temperature for 2 h.

For electrophoresis, discontinuous 12% SDS pre-casted acrylamide gels from Invitrogen was used. All samples were boiled with lithium dodecyl sulphate (LDS) sample buffer for 5 min. Gels were run at a constant voltage of 200 V for approximately 1 h. After transfer onto PVDF membranes (30 V for 2 h), the membranes were incubated for 1 h with 1 μg/mL of R&D antibody. After washing, the secondary anti-mouse antibody labelled with alkaline phosphatase was then added and incubated for 1 h. Chemiluminescent substrate was then blotted over the membrane for about 5 min. X-ray film was used for luminography and to visualize the bands (15–25 min exposure). Protein standard (Ladder) was used to visualize the reference standard bands (MagicMark cat. no. LC5602). Recombinant human IGFBP-1 (rhIGFBP-1) (R&D cat. no. 871-B1) was used as a reference sample of non-phosphorylated IGFBP-1 (npIGFBP-1). To confirm that R&D capture antibody binds to npIGFBP-1 and/or less-phosphorylated IGFBP-1 (lpIGFBP-1) normal serum sample showed both bands when applied as in Fig. 1 (lane 3). When phosphoserine-containing proteins in a serum sample were immunoprecipitated using an antiphosphoserine antibody (BD cat no. 612547) (Fig. 1, lane 4) this resulted in the appearance of a pIGFBP-1 isoform alone that could be detected by R&D antibody. Serum dephosphorylation with alkaline phosphatase resulted in the appearance of an npIGFBP-1 band alone (Fig. 1, lane 5). rhIGFBP-1 which is non-phosphorylated served as a reference material (Fig. 1, lane 1).

IGFBP-1 ELISA protocol

IGFBP-1 was measured using an in-house ELISA with maximum analytical CVs of 7.0% and 9.1% for intra- and inter-

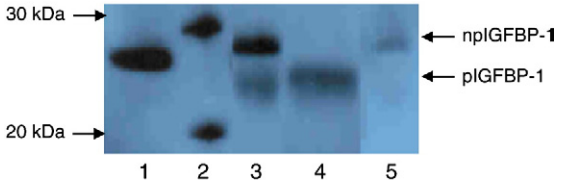

Fig. 1. IGFBP-1 Western ligand blotting using R&D monoclonal antibody: 1. rhIGFBP-1 2. Ladder (molecular weight of 20 and 30 kDa) 3. Normal serum sample (x) 4. (x) following precipitation by antiphosphoserine antibody. 5. (x) after pretreatment with alkaline phosphatase.