Endocrine, *vol. 5, no. 1, 1–8, August 1996* 1046–3976/5/1:1–8/$7.00

Collection of Blood in Heparinized Tubes Does Not Alter the Molecular Distribution or Forms of IGFBP-3 and IGF

Scott Mandel, Erin Moreland, Ron G. Rosenfeld, and Sharron E. Gargosky

Department of Pediatrics, Oregon Health Sciences University, Portland, OR

The major serum carrier of the insulin-like growth factors (IGFs) is IGF-binding protein-3 (IGFBP-3) that exists in the circulation associated with IGF and an acid labile subunit to form a ternary (158-kDa) complex. It has been reported that heparin disrupts the IGF carrying capacity of the ternary complex and is a potent inhibitor of ternary complex reformation (Clemmons et al., 1983; Baxter, 1990). Thus, the aim of this study was to determine if, in a clinical setting where blood may be collected in both nonheparinized and heparinized tubes, heparin alters the molecular distribution or immunoreactive measurement of IGFBP-3 and IGF-I. Two different collection modalities were examined: protocol 1, blood was drawn and immediately centrifuged and aliquotted; and protocol 2, blood was drawn, left at room temperature for 2 h and then at 4°C overnight prior to centrifugation. Samples were drawn from a normal adult and from a growth hormone-deficient (GHD) child and subjected to neutral size-exclusion chromatography to separate the ternary 158-kDa complex from the binary IGFBP-3-IGF (approx 50 kDa) complex. Fractions were then subjected to Western ligand blot (WLB), western immunoblot (WIB), and measurement of IGFBP-3 by immunoradiometric assay (IRMA), while the IGF distribution was measured by radioimmunoassay (RIA) following acidic size-exclusion chromatography. In both serum and plasma of a normal adult, WLB detected a 45–40-kDa IGFBP-3 doublet eluting primarily within the 158-kDa IGFBP region (i.e., ternary complex). Similarly, assessment of immunoreactive IGFBP-3 by WIB showed a 45–40-kDa IGFBP-3 doublet, as well as a 29 kDa immunoreactive form primarily eluting in the 158-kDa IGFBP region of the chromatograph. Measurement of IGFBP-3 by IRMA confirmed these findings. No difference between serum and plasma was detected in either collection protocol. RIA of IGF-I revealed that the ternary complex carried the majority of the circulating IGF-I and that there was no difference between serum and plasma. Assessment of serum and plasma of a GHD child showed reduced serum concentrations of IGFBP-3 but no difference in the IGFBP profiles between serum and plasma. These data demonstrate that the collection of blood in heparinized tubes does not alter the molecular distribution or forms of IGFBP-3 and IGF-I.

Key Words: IGF-I; IGFBP-3; ALS; plasma; heparin.

Introduction

The somatomedins have been structurally recognized to belong to the insulin family and are now referred to as the IGFs that are GH-, age-, and nutritionally-dependent. These potent mitogens are transported complexed to IGF-binding proteins (IGFBPs), which have been shown to increase the half-lives of IGFs and to modulate their bioavailabilities and bioactivities (reviewed: Baxter 1988a; Baxter and Martin, 1989a; Holly and Wass, 1989; Rosenfeld et al.,1991; Shimazaki and Ling, 1991; Kelley et al., 1996). Six IGFBPs have been classified (Ballard et al., 1990, 1992). The predominant serum IGFBP is GH-dependent IGFBP-3, a glycosylated protein of 45 kDa that circulates as a large 158-kDa ternary complex associated with IGF and an acid-labile subunit (Baxter and Martin 1989b; Baxter, 1988b, 1990a). In adult nonpregnancy human serum, the 158-kDa IGFBP complex carries 70–90% of the total circulating IGF, with a linear relationship between serum concentrations of IGFBP-3 and IGF peptides (Bang et al., 1993; Daughaday and Rotwein, 1989; Gargosky et al., 1992; Hardouin et al., 1989).

The clinical interest in these growth factors and their circulating carriers is becoming widespread as a result of their potential clinical application and diagnostic potentials (Froesch et al., 1990; Muller et al., 1994; Clemmons and Underwood, 1994; Rosenfeld et al., 1995). However, a potential complication is the evidence that heparin can inhibit formation of the ternary complex as well as potenti-

Received February 14, 1996; Revised April 10, 1996; Accepted April 10, 1996.
Author to whom all correspondence and reprint requests should be addressed: S. Mandel, Department of Pediatrics, NRC5, Oregon Health Sciences University, 3181 SW Sam Jackson Park Rd, Portland, OR.

ate dissociation of IGFs from their IGFBPs (Clemmons et al., 1983; Baxter 1990a). Thus, the aim of this study was to collect blood in both heparinized and nonheparinized collection tubes and analyze the distribution of IGFBP-3 and its ability to maintain ternary complex formation, the immunoreactive forms of IGFBP-3, and the distribution of IGF-I.

Methods and Materials

Specimen Collection

Blood from a normal adult was drawn into two 5-mL glass tubes containing 72 USP units heparin/tube, as well as two tubes with no additive, and processed as follows. Protocol A: One of each type was centrifuged immediately at 1800 rpm room temperature, yielding plasma and serum that were then frozen at –70 C. This treatment represents an ideal clinical condition for the handling of blood samples. The other set of heparinized/nonheparinized tubes was allowed to incubate 2 h at room temperature, and then overnight at 4°C, before being centrifuged and frozen (Protocol B). We considered this a less favorable treatment of the sample, which would provide more time for heparin to affect the ternary IGFBP-3 complex, if indeed the heparin was to have a measurable effect. Although an individual subject was used, the blood samples were drawn on at least two different occasions over the course of six months, and the data sets were identical. Further, each analysis was performed multiple times with the maximal intersample variation < 10%.

Blood was also drawn from a prepubertal growth hormone deficient patient who responded to an insulin-arginine stimulation test with a GH peak of 4.1 ng/L. The plasma and serum were prepared according to Protocol A only.

Peptides and Antiserum

Recombinant human IGF-I for radioimmunoassay was provided by Bachem (Torrance, CA). IGF-II was provided by Eli Lilly Research Laboratories (Indianapolis, IN). Both peptides were iodinated by a modification of the chloramine T method (Van Obberghen-Schilling and Pouyssegur, 1983) to specific activities of 350–500 μCi/μg.

Anti-IGF-I rabbit antiserum (UB3–189) was a gift of Drs. Louis Underwood and Judson J. Van Wyk, Division of Pediatric Endocrinology, University of North Carolina at Chapel Hill, and was distributed for research use by the Hormone Distribution Program of NIDDK through the National Hormone and Pituitary Program. IGFBP-3 antiserum used for WIB analysis was generated in our laboratory (Gargosky et al., 1992).

Neutral Size-Exclusion Fast Performance Liquid Chromatography

Two hundred microliters from each plasma and serum sample was delipidated by extraction with an equal volume of freon (1,1,2-trichloro-1,2,2-trifluoroethane). Isolation of the 158-kDa ternary (ALS-IGFBP-3-IGF) complex from the 44 kDa binary (IGFBP-3-IGF) complex was achieved via chromatography through a Superose-12 column, using a Pharmacia FPLC system in buffer (50 m*M* $NaH2PO_4$, 158 m*M* NaCl, 0.02% Na azide, pH 7.4) with a flow rate of 0.5 mL/min. The column was precalibrated with gel filtration standards: thyroglobulin, 670 kDa; gamma globulin, 158-kDa; ovalbumin, 44 kDa; myoglobin, 17-kDa; and vitamin B-12, 1.35 kDa (BioRad Laboratories, Richmond, CA). Iodinated IGF-I, 7-kDa, and bovine serum albumin, 69 kDa, were also used in column calibration. The 0.5-mL fractions from plasma and serum were frozen at –70°C before their use in the assays and Western analysis.

IGFBP-3 Quantitation

Fractions of chromatographed plasma and serum spanning the 158- and 44-kDa regions of each sample, as well as the corresponding whole plasma and serum, were assayed for IGFBP-3 using immunoradiometric assay (IRMA) kits generously supplied by Diagnostics Systems Laboratories (Webster, TX).

Western-Ligand Blot Analysis

Fifty microliters of each FPLC fraction was subjected to Western-ligand blot analysis, as described by Hossenlopp et al. (1989). Samples were diluted with nonreducing SDS-dissociation buffer (0.5*M* Tris, pH 6.8; 69% glycerol; 4% sodium dodecylsulphate), loaded onto a 1-mm discontinuous SDS polyacrylamide gel and electrophoresed through a 4% stacking gel and 10% separating gel (Laemmli, 1970) at 50 V overnight. Molecular weight markers in SDS-dissociation buffer containing 1 m*M* dithiothreitol as a reductant were also electrophoresed. Proteins were then electrotransfered from gels to 0.45 μnitrocellulose (Schneider and Schuell) at 0.2 A for 1.5 h with a Hoefer Semi-dry Transphor unit (San Francisco, CA), following the method of Towbin et al. (1979). The nitrocellulose-bound proteins were then treated with 3% NP40 for 30 min. and 1% BSA for 2 h. Each treatment was carried out at room temperature, in a Tris-saline buffer (0.1*M* Tris, 0.15*M* NaCl; pH 7.4). The treated nitrocellulose filters were probed with radiolabeled IGF-II (one million cpm/filter) overnight. They were then washed extensively in 1% Tween-20, air-dried, and exposed to X-ray film (Kodak X-Omat AR) in the presence of Cronex Hi-Plus Intensifying Screens (Du Pont) for 4 d at –70°C.

Western-Immunoblot Analysis

Samples were subjected to nonreducing gel electrophoresis and electrotransferred as described in the above paragraph. After being blocked with 1% BSA for 4 h in a buffer containing 0.1*M* Tris and 0.15*M* NaCl at pH 7.4, the nitrocellulose was incubated overnight with IGFBP-3 antiserum, αIGFBP-3g1 (Gargosky et al., 1992) in the same

1% BSA saline buffer. The nitrocellulose was then washed two times for 10 min in 0.1% Tween-20. Following a 2 h incubation with goat antirabbit IgG conjugated with horseradish peroxidase, the nitrocellulose was washed again in 0.1% Tween-20 buffer (2 × 10 min), treated with 10 mL each of enhanced chemiluminescence reagents (Amersham, Arlington Heights, IL) for 1 min, rinsed quickly with 0.1% Tween-20, and exposed to X-ray film for 1 h.

IGF-I Quantitation

FPLC fractions spanning the 158- and 44-kDa regions of each sample, as well as the corresponding whole plasma and serum, were subjected to size-exclusion chromatography through a Sephadex G-50 (fine) column (Pharmacia, Piscataway, NJ) in 1% formic acid. The dissociated IGFs eluted between 43 and 80 mL, and were collected in tubes containing 1 mL of 1% BSA in Tris-saline solution. The eluates were then lyophilized and reconstituted to a dilution of 1/10 the original volume in radioimmunoassay buffer (0.04*M* $NaH2PO_4$, 0.15*M* NaCl, 0.5% BSA, 0.1% Na azide; pH 7.4). IGF-I was measured by RIA, as previously described by Powell et al. (1986). The minimal detectable concentration was 0.1 ng/mL.

Results

We have previously shown that neutral size-exclusion chromatography separates the serum IGF-IGFBP complexes into three major regions: a 158-kDa IGFBP region that comprises IGFBP-3, a 60–40-kDa IGFBP region that contains some IGFBP-3 as well as smaller IGFBPs, and a 7-kDa uncomplexed IGF region (Gargosky et al., 1990, 1993). In this study, to determine the distribution of immunoreactive IGFBP-3 between the 158-kDa and smaller IGFBP regions, serum and plasma were fractionated under neutral conditions and assessed by IRMA for IGFBP-3. Blood was collected and treated as described in Methods and Materials: protocol A (immediately centrifuged and aliquotted) or protocol B (incubated 2 h at room temperature, then at 4°C overnight prior to centrifugation and aliquotting) (Fig. 1). As expected, two major IGFBP regions were measured in both serum and plasma treated as in protocol A, showing a peak of immunoreactivity at fraction 23 within the 158-kDa region and at fraction 28 within the 60–40-kDa region of the chromatograph (data not shown). Summation of fractions 19–27.5 created the 158-kDa IGFBP region, and fractions 27.5–32 created the 60–40-kDa IGFBP region. Comparing serum and plasma treated as in protocol A revealed no difference between the concentrations of IGFBP-3 measured in the 158- and 60–40-kDa regions (Fig. 1A). Similarly, there was no difference in the immunoreactivity measured in serum and plasma treated as in protocol B (Fig. 1B). Thus, neither heparinization of blood nor extended incubation of blood with heparin affected the distribution of immunoreactive IGFBP-3, as assessed by IRMA. Subsequently, we used only serum and plasma treated as in protocol B, the less favorable scenario, to assess the effect of heparin on the distribution and molecular forms of IGFBPs and IGFs (Table 1).

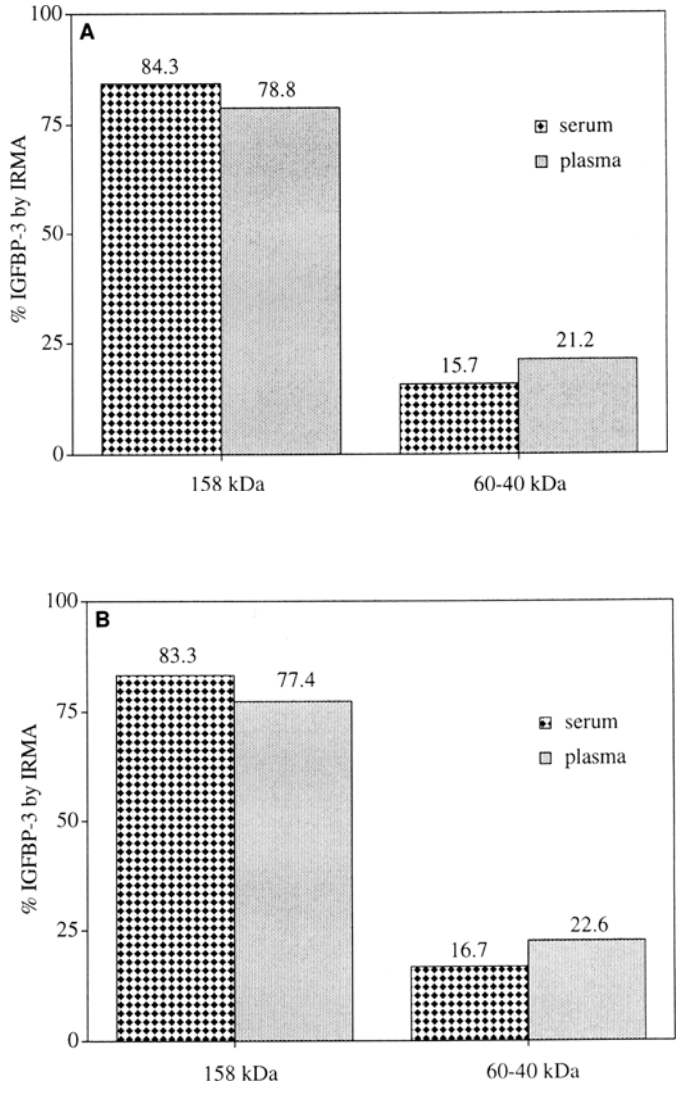


Fig 1. Molecular distribution of IGFBP-3 in serum and plasma collected from a normal adult. Blood was drawn into two sets of nonheparinized and heparinized (72 USP units) 5 mL tubes. One set **(A)** was centrifuged immediately at 1800 rpm room temperature, yielding serum and plasma which were then frozen at –70 C. The other set of nonheparinized and heparinized tubes **(B)** was allowed to incubate 2 h at room temperature, and then overnight at 4°C, before centrifugation and freezing of serum and plasma. Two hundred microliters from each specimen was freon-extracted and subjected to neutral size-exclusion chromatography over a Pharmacia Superose-12 column, separating the ternary ALS-IGFBP-3-IGF complex from the binary IGFBP-3-IGF complex. The resulting 0.5-mL fractions were assayed for their IGFBP-3 content using IRMA kits supplied by DSL. As determined by precalibration of the S-12 column with molecular weight standards, fractions 19–27.5 were considered to contain ternary complex, while fractions 27.5–32 represent the binary complex. IGFBP-3 levels within the respective regions were summed, and graphs show the percentage of the total IGFBP-3 measured in each region. This is a representative data set.

Table 1
Summation of IGF-I and IGFBP-3

Sample	IGF-I ng/mL RIA	IGFBP-3 ng/mL IRMA
Adult human serum	183	2924
Adult human plasma	149	3721
Case B human plasma	186	3632
Case B human serum	174	3820
GHD serum	43	1151
GHD plasma	43	1271

[a]Serum and plasma were subjected to an IGF-I RIA following acid size-exclusion chromatography and to an IGFBP-3 IRMA. Table shows serum and plasma values were comparable for a normal adult specimen processed immediately (Case A), and after a 2 h room temperature and then overnight 4°C incubation prior to centrifugation of collection tubes (Case B). Similarly, serum and plasma values were comparable for a growth hormone-deficient child.

To evaluate the molecular form of IGFBP-3 in both serum and plasma, following size-exclusion chromatography fractions were subjected to WLB to assess the ability of IGFBPs to bind ligand (Fig. 2), and WIB using an antiserum against human IGFBP-3 to examine the immunoreactive forms of IGFBP-3 (Fig 3).

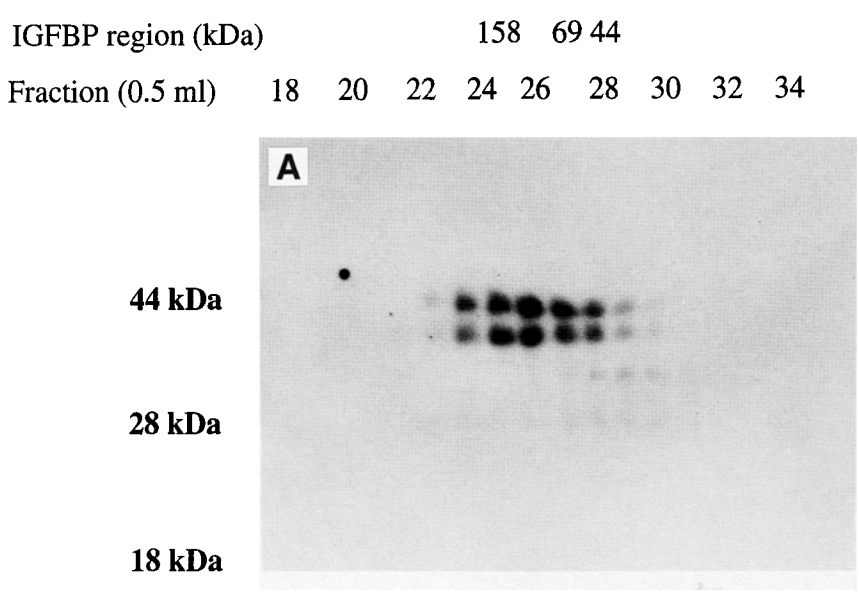
Serum from a normal healthy adult revealed a 45–40-kDa IGFBP doublet eluting predominantly in the 158-kDa IGFBP region (fractions 23–26). Based on electrophoretic migration and the elution of the doublet within the chromatograph, this doublet is most likely IGFBP-3. Similarly, the smaller IGFBPs most likely represent the 34-kDa IGFBP-2 and the 28-kDa IGFBP-1 which were detected in the 60–40-kDa IGFBP region of the chromatograph (Fig 2A). Plasma from the same healthy adult produced a similar IGFBP profile with a 45–40-kDa IGFBP-3 doublet eluting within the 158-kDa IGFBP region, while smaller IGFBPs were detected in the 60–40-kDa IGFBP region (fraction 27–29) (Fig 2B). Densitometric analysis of the 45–40-kDa IGFBP-3 doublet revealed no difference between serum and plasma in the intensity of the bands in either IGFBP region (Fig. 2C).

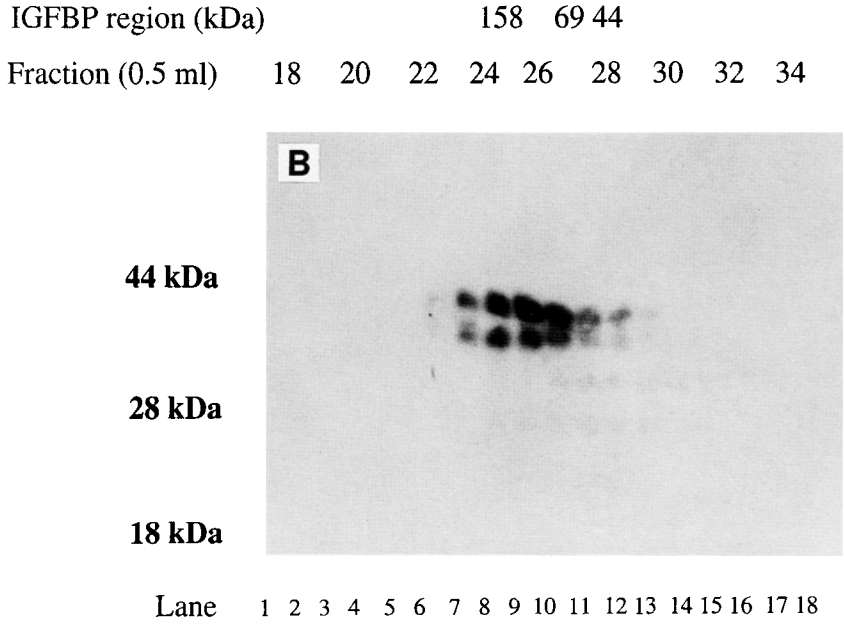
It has been extensively documented that IGFBP-3 exists in normal serum as two predominant immunoreactive forms and that the ratio of these forms may be influenced by IGFBP proteases (Gargosky et al., 1992). Thus, to ensure that the 45–40-kDa doublet was in fact IGFBP-3, and determine which immunoreactive forms of IGFBP-3 existed within serum and plasma, the same sheets of nitrocellulose were subjected to WIB analysis using a specific antibody against human IGFBP-3 (Fig. 3). WIB analysis of fractionated normal adult serum (Fig. 3A) and plasma (Fig. 3B) revealed the 45–40-kDa IGFBP-3 doublet as well as a 28-kDa IGFBP-3 within the 158-kDa IGFBP region of the chromatograph. Note that the 28-kDa band did not bind iodinated IGF ligand (Fig. 2), and was only detected by immunoreactivity. Densitometric analysis of both immunoreactive forms of IGFBP-3 in serum or plasma showed no difference in band intensity within the 158-kDa IGFBP region (Fig. 3C). In addition, densitometric analysis of the 45–40-kDa doublet and the 28-kDa IGFBP-3 fragment showed no differences in their distribution or elution profile. Thus, heparinization of blood neither altered the IGFBP profile as assessed by WLB, nor changed the distribution or molecular forms of IGFBP-3.

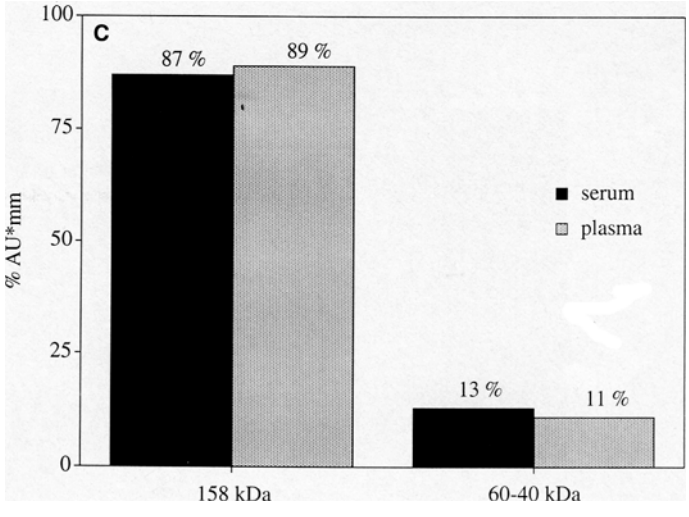
Finally, to determine if the concentration of IGF-I carried by the IGFBP differed between serum and plasma, samples were first subjected to neutral size-exclusion chromatography to define the IGFBP regions and then the fractionated samples were individually rechromatographed under acidic size-exclusion chromatography to separate the IGFBPs from the IGFs. Circulating IGF-I was then measured by RIA and expressed as a percentage of the total recovered IGF (Fig. 4). An average of 60% of the total circulating IGF-I was measured within the 158-kDa ternary complex, a level similar to the measurement of immunoreactive IGFBP-3 measured within this same IGFBP region (Fig 1). A small amount of IGF-I was detected within the 60–40-kDa IGFBP region and in the 7-kDa region. Again, there was no difference between serum and plasma in their IGF distribution.

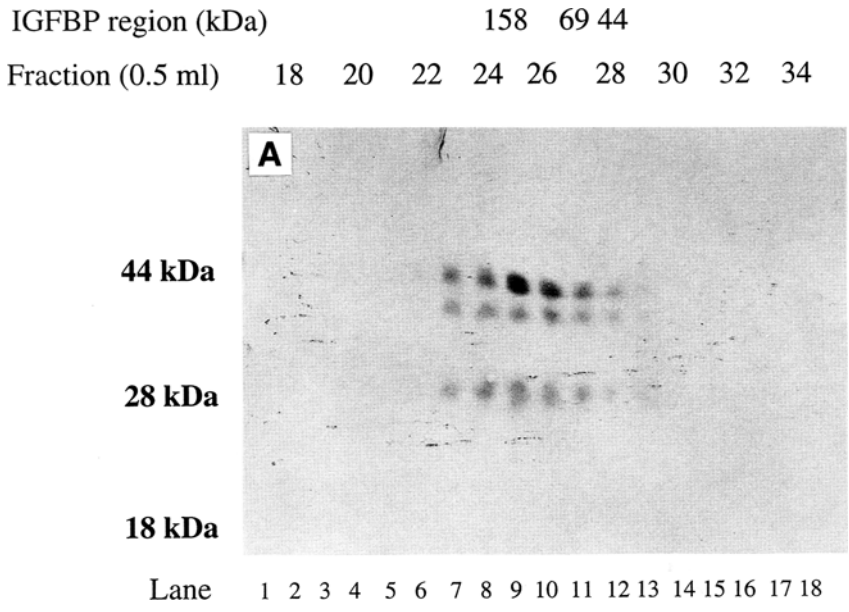
Since the IGFs and particularly IGFBP-3 are receiving widespread attention as a diagnostic marker for GHD we decided to assess the effect of heparin on the IGFBP profile of a GHD child. Serum and plasma were fractionated under neutral conditions and IGFBP-3 measured by IRMA. No difference between serum or plasma was detected in the total IGFBP-3 measured or in the distribution of IGFBP-3; although the absolute amount of IGFBP-3 was 50% that of a healthy adult.

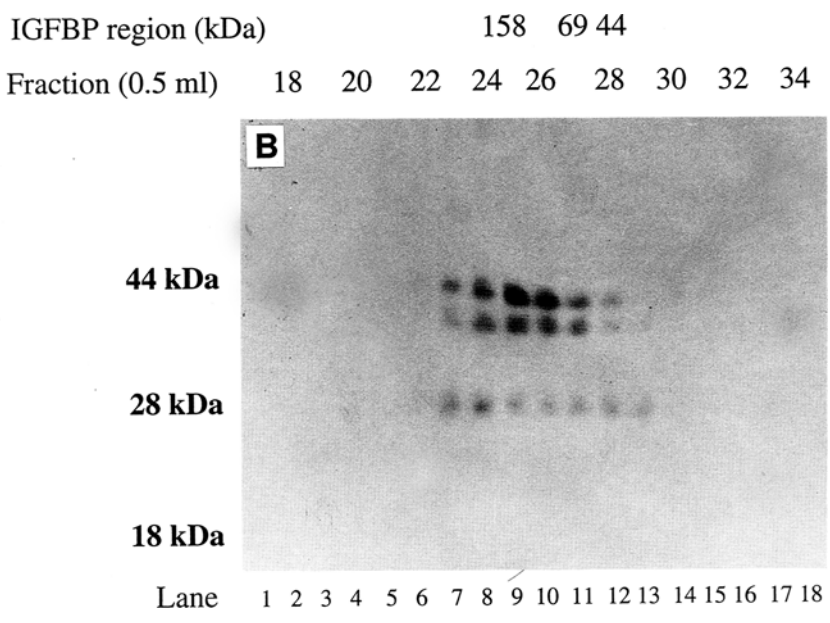
Fig. 2. *(opposite page)* Characterization by Western-ligand blot of the IGFBP regions. Normal adult serum and plasma collected after blood was allowed to incubate 2 h at room temperature, and then overnight at 4°C, in nonheparinized and heparinized tubes. Following freon-extraction and neutral size-exclusion chromatography over a Superose-12 column into 0.5-mL fractions, 50 μL from each serum **(A)** and plasma **(B)** fraction was subjected to WLB analysis, probed with iodinated IGF-II, and autoradiographed for 4 d at –70 C. The molecular weight regions separated through neutral size-exclusion chromatography are indicated across the top of the panel: 158- and 44-kDa. The molecular weight markers electrophoresed through the gel are indicated on the left side of the panel. Serum and plasma show similar profiles, with a 45–40-kDa IGFBP-3 doublet eluting within the 158-kDa region and smaller IGFBPs detected in the 60–40-kDa IGFBP region (fractions 27–29). Densitometric analysis revealed no difference between serum and plasma in the proportion of IGFBP-3 in either region.

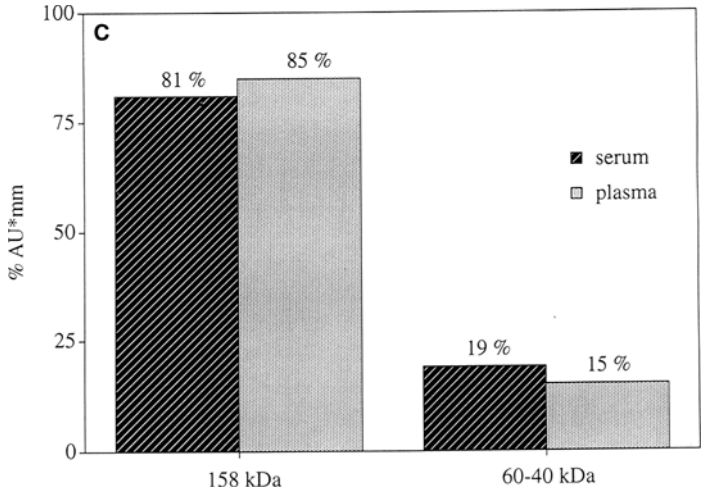

IGFBP region (kDa)
158 69 44
Fraction (0.5 ml)
18 20 22 24 26 28 30 32 34
A
44 kDa
28 kDa
18 kDa
Lane
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18


IGFBP region (kDa)
158 69 44
Fraction (0.5 ml)
18 20 22 24 26 28 30 32 34
B
44 kDa
28 kDa
18 kDa
Lane
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18


C
100
75
50
25
0
% AU*mm
87 %
89 %
13 %
11 %
serum
plasma
158 kDa
60-40 kDa


IGFBP region (kDa)
158 69 44
Fraction (0.5 ml)
18 20 22 24 26 28 30 32 34
A
44 kDa
28 kDa
18 kDa
Lane
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18


IGFBP region (kDa)
158 69 44
Fraction (0.5 ml)
18 20 22 24 26 28 30 32 34
B
44 kDa
28 kDa
18 kDa
Lane
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18


C
100
75
50
25
0
% AU*mm
81 %
85 %
19 %
15 %
serum
plasma
158 kDa
60-40 kDa

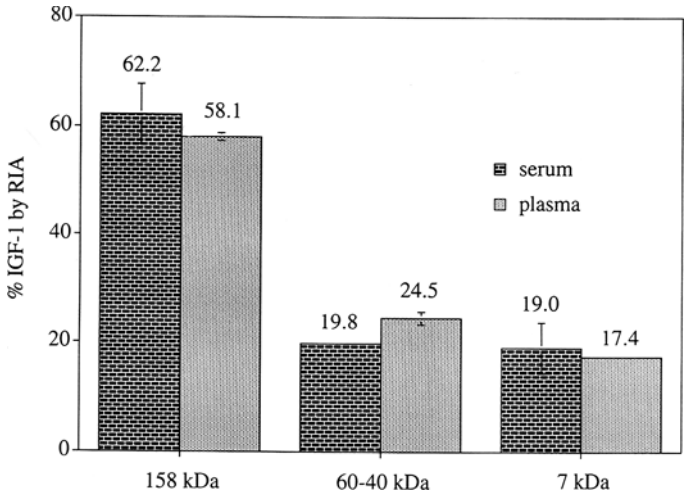


Fig. 4. Distribution of IGF-I in serum and plasma. Samples were collected following protocol B and subjected to neutral size-exclusion chromatography over a Superose-12 column. Fractions spanning the 158- and 44-kDa regions of each sample were chromatographed further through a Sephadex G-50 (fine) column in 1% formic acid, in order to dissociate the IGFs from their binding proteins. IGF-I levels in each fraction were measured twice via RIA, and grouped according to IGF-I region (158-, 60–40-, and 7-kDa). Results within each group were summed and expressed as a percentage (± SEM) of the total IGF-I recovered. There was no significant difference between serum and plasma in their IGF-I distribution.

Western-ligand blot of serum from a GHD child and subsequent densitometric analysis showed similar proportions of the 45–40-kDa IGFBP-3 doublet within the 158-kDa region of the chromatograph in serum vs plasma. WIB using a specific IGFBP-3 antiserum showed a 45–40-kDa IGFBP-3 doublet and a smaller 28-kDa form of IGFBP-3 primarily within the 158-kDa region of the chromatograph. Densitometric analysis of both the IGFBP-3 doublet and fragment showed no difference in the distribution between the ternary and binary complexes in serum vs plasma. These data further support the finding that heparinization of blood does not affect the distribution of IGFBPs in a child with GHD and lower levels of serum IGF-I and IGFBP-3.

Discussion

It has been documented that heparin may liberate 70–80% of circulating IGF from the ternary complex to an uncomplexed form (Clemmons et al., 1983) and more recently that heparin is a potent inhibitor of complex formation (Baxter, 1990a). Thus, the use of heparin as an anticoagulant in the collection of plasma may alter the assessment of the IGF axis. To address this potential problem we analyzed the distribution of IGF among the IGFBP-3 complexes. In addition, blood was collected by two different protocols; one representing an ideal scenario where blood was collected, centrifuged, and aliquotted immediately; whereas the second was a less favorable scenario of a room temperature incubation followed by incubation at 4°C overnight prior to analysis.

We did not find any change in the distribution of circulating IGF-I. In agreement with previous reports that have evaluated serum (Baxter and Martin, 1986; Gargosky, 1991; Gargosky et al., 1993), we measured 60% of the total circulating IGFs associated with the 158-kDa ternary complex of normal healthy adults. There was no difference between serum and plasma in the IGF-I measured in the ternary complex. It is of note that Clemmons et al. (1983) also showed no difference between heparinized plasma and serum until these samples were extensively incubated at elevated temperatures (37°C for 24 h), at which time heparin released IGF from the IGFBPs. We have examined the molecular form and distribution of IGFBPs, focusing on the predominant serum carrier of IGFs, IGFBP-3. Neutral size-exclusion chromatography combined with WLB and WIB revealed no differences between serum and plasma. IGFBP-3 eluted primarily within the 158-kDa IGFBP region, i.e., within the ternary complex. We detected two immunoreactive forms of IGFBP-3 within the ternary complex region one of which was not detectable by WLB (Gargosky et al., 1992; Bang et al., 1994). Suikkari and Baxter (1991) have shown that the 28-kDa form of IGFBP-3 can bind IGF, but not iodo-IGF. Further, Liu et al. (1992) have shown that in pregnancy serum, a 28-kDa form of IGFBP-3 is able to reassociate with IGF and ALS and form the 150–130-kDa complex. These data are consistent with the 28-kDa IGFBP-3 detected in our serum and plasma, which is highly

Fig. 3. *(previous page)* Characterization by Western-immunoblot of the molecular forms of IGFBP-3. Normal adult serum and plasma collected after blood was allowed to incubate 2 h at room termperature, and then overnight at 4°C, in nonheparinized and heparinized tubes. Following freon extraction and neutral size-exclusion chromatography over a Superose-12 column, 50 µL from each serum **(A)** and plasma **(B)** fraction was subjected to WIB analysis using antisera against IGFBP-3 and the Enhanced Chemiluminescence detection system, and the nitrocellulose was exposed to X-ray film for 1 h. The molecular weight regions separated through neutral size-exclusion chromatography are indicated across the top of the panel, and the molecular weight markers electrophoresed through the gel are indicated on the left side of the panel. Serum and plasma exhibit similar profiles of the 45–40-kDa IGFBP-3 doublet as well as a 28-kDa IGFBP-3 within the 158-kDa IGFBP region of the chromatograph. Densitometric analysis of both immunoreactive forms of IGFBP-3 revealed no significant difference between serum and plasma in the proportion of IGFBP-3 found in the 158- vs the 44-kDa region.

immunoreactive and part of the ternary complex, yet binds radiolabeled IGF poorly. However, its significance as part of the 150 kDa complex is uncertain. Interestingly, the proportion of the 45–40-kDa doublet of IGFBP-3 to the 28-kDa immunoreactive form of IGFBP-3 was not different between serum and plasma, further indicating that the level of heparin in plasma collection tubes does not influence the immunoreactive forms of IGFBP-3 or the distribution of IGFBP-3. This was further supported by immunoradiometric assay data that measured equivalent proportions of IGFBP-3 in the ternary complex of serum and plasma.

We also examined the IGFBP profile of a GHD child since IGFBP-3 levels are extensively reported to be lower (Baxter 1986, 1988; Mandel et al., 1995; Rosenfeld et al., 1995) and assessed whether heparin could affect the molecular forms or distribution of IGFBP-3. We found no difference in the immunoreactive profile, WLB, or WIB analysis when comparing serum vs plasma.

In conclusion, collection of blood in heparinized tubes does not alter the molecular forms of IGFBP-3 or the distribution of IGF-I among the IGFBPs.

Acknowledgments

Supported in part by a grant from the Genentech Foundation.

References

Ballard, J., Baxter, R., Binoux, M., Clemmons, D., Drop, S., Hall, K., Hintz, R., Rechler, M., Rutanen, E., and Schwander, J. (1990). *Acta Endocrinologica* **121,** 751,752.

Ballard, F. J., Baxter, R. C., Binoux, M., Clemmons, D. R., Drop, S. L. S., Hall, K., Hintz, R. L., Rechler, M. M., Rutanen, E. M., Schwander, J. C., Spencer, E. M., and Zapf, J. (1992). *J. Clin. Endocrin. Metab.* **74,** 1215,1216.

Bang, P., Degerbled, M., Thoren, M., Schwander, J., Blum, W., and Hall, K. (1993). *Acta Endocrinol.* **128,** 397–404.

Bang, P., Brismar, K., and Rosenfeld, R. G. (1994). *J. Clin. Endocrin. Metab.* **78,** 1119–1127.

Baxter, R. C. and Martin, J. L. (1986). *J. Clin. Invest.* **78,** 1504–1512.

Baxter, R. C. (1988a). *Comp. Biochem. Physiol.* **91B,** 229–235.

Baxter, R. C. (1988b). *J. Clin. Endocrin. Metab.* **67,** 265–272.

Baxter, R. C. (1990a). *Biochem. J.* **271,** 773–777.

Baxter, R. C. (1990b). *J. Clin. Endocrin. Metab.* **70,** 1347–1353.

Baxter, R. C. and Martin, J. L. (1989a). *Prog. Growth Factor Res.* **1,** 49–68.

Baxter, R. C. and Martin, J. L. (1989b). *Proc. Natl. Acad. Sci. USA* **86,** 6898–6902.

Clemmons, D. R., Underwood, L. E., Chatelain, P. G., and Van Wyk, J. J. (1983). *J. Clin. Endocrin. Metab.* **56,** 384–389.

Clemmons, D. R. and Underwood, L. E. (1994). *J. Clin. Endocrin. Metab.* **79,** 4–6.

Daughaday, W. H. and Rotwein, P. (1989). *Endocrine Rev.* **10,** 68–91.

Froesch, E. R., Guler, H. P., Schmidt, C., Binz, K., and Zapf, J. (1990). *TEM.* **May/June,** 254–260.

Gargosky, S. E., Walton, P. E., Wallace, J. C., and Ballard, F. J. (1990). *J. Endocrinol.* **127,** 291–300.

Gargosky, S. E. (1991). PhD Dissertation. University of Adelaide, SA, Australia.

Gargosky, S. E., Pham, H. P., Wilson, K. F., Liu, F., Giudice, L. C., and Rosenfeld, R. G. (1992). *Endocrinology* **131,** 3051–3060.

Gargosky, S. E., Wilson, K. F., Fielder, P. J., Baxter, R. C., Vacarello, M. A., Diamond, F. B., Guevara-Aguirre, J., Rosenbloom, A., and Rosenfeld, R. G. (1993). *J. Clin. Endocrinol. Metab.* **77,** 1683–1689.

Hardouin, S., Gourmelen, M., Noguiez, P., Seurin, D., Roghani, M., Le Bouc, Y., Povoa, G., Merimee, T. J., Hossenlopp, P., and Binoux, M. (1989). *J. Clin. Endocrinol. Metab.* **69,** 1291–1301.

Holly, J. M. P. and Wass, J. A. H. (1989). *J. Endocrin.* **122,** 611–618.

Hossenlopp, P., Seurin, D., Segovia-Quinson, B., Hardouin, S., and Binoux, M. (1986). *Anal. Biochem.* **154,** 138–143.

Kelley, K. M., Oh, Y., Gargosky, S. E., Gucev, Z., Matsumoto, T., Hwa, V., Ng, L., Simpson, D., and Rosenfeld, R. G. (1996). *Int. J. Biochem.* **28,** 619–637.

Laemmli, U. K. (1970). *Nature* 227, 680–685.

Liu, F., Baxter, R. C., and Hintz, R. L. (1992). *J. Clin. Endocrin. Metab.* **75,** 1261–1267.

Mandel, S., Moreland, E., Nichols, V., Hanna, C., and LaFranchi, S. (1995). *J. Clin. Endocrin. Metab.* **80,** 190–194.

Muller, H. L., Oh, Y., Gargosky, S. E., Lernbacher, T., Hintz, R. L., and Rosenfeld, R. G. (1994). *J. Clin. Endocrin. Metab.* **77,** 1113–1119.

Powell, D. R., Rosenfeld, R. G., Baker, B., Liu, F., and Hintz, R. L. (1986). *J. Clin. Endocrinol. Metab.* **63,** 1186–1192.

Rosenfeld, R. G., Lamson, G. L., Pham, H., Oh, Y., Conover, C., DeLeon, D. D., Donovan, S. M., Ocrant, I., and Giudice, L. C. (1991). *Recent Prog. Hormone Res.* **46,** 99–159.

Rosenfeld, R. G., Albertson-Wikland, K., Cassorla, F., Frasier, S. D., Hasegawa, Y., Hintz, R. L., LaFranchi, S., Lippe, B., Loriaux, L., Melmed, S., Preece, M. A., Ranke, M. B., Reiter, EO., Rogol, A. D., Underwood, L. E., and Werther, G. A. (1995). *J. Clin. Endocrin. Metab.* **80,** 1532–1540.

Shimazaki, S. and Ling, N. (1991). *Prog. Growth Factor Res.* **3,** 243–266.

Suikkari, A. M. and Baxter, R. C. (1991). *J. Clin. Endocrinol. Metab.* **73,** 1377–1379.

Towbin, H., Staehlin, T., and Gordin, J. (1979). *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.

Van Obberghen-Schilling, E. and Pouyssegur, J. (1983). *Exp. Cell Biol.* **151,** 207–214.