MINIREVIEW

Regulation and Function of Insulin-Like Growth Factor-Binding Protein-1 (43630)

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Abstract. **Insulin-like growth factor-binding protein (IGFBP)-1 is one of six structurally homologous proteins that specifically bind and modulate the mitogenic and metabolic actions of insulin-like growth factor (IGF)-I and IGF-II. Of the six IGFBP, IGFBP-1 is the only one that displays rapid dynamic regulation *in vivo*, with serum levels varying 10-fold or more in relation to meals.**

The complementary cDNA for IGFBP-1 was first reported in 1988. The predicted 234-amino acid sequence has a molecular mass of 25.3 kDa. The N-terminal and C-terminal regions are highly homologous among rat, human, and bovine sequences, and contain 18 conserved cysteines which are postulated to provide a framework for ligand binding. The 65-residue midregion is less homologous and does not contain cysteines, but does include a Pro-Glu-Ser-Thr (PEST) domain that is typical of rapidly metabolized proteins. The gene for IGFBP-1 has been localized to human chromosome region 7p12-p14, where it is contiguous with the gene for IGFBP-3.

IGFBP-1 mRNA and protein expression have been identified in human liver and uterine decidua, and in nonhuman kidney. *In vitro* and *in vivo* studies indicate that insulin is the primary regulator of IGFBP-1 expression in these tissues, and that the primary effect of insulin is rapid inhibition of transcription. On the other hand, cortisol, glucagon, and cAMP stimulate IGFBP-1 production. Limited data also show a potent stimulatory effect of phorbol esters. A detailed review of IGFBP-1 levels and physiology *in vivo* and *in vitro* is presented.

The function of IGFBP-1 is not completely defined. However, several studies demonstrate that IGFBP-1 inhibits IGF binding to cell surface receptors and thereby inhibits IGF-mediated mitogenic and cell metabolic actions. Furthermore, IGFBP-1 regulation by insulin and glucoregulatory hormones *in vitro* and limited *in vivo* data are consistent with a role for IGFBP-1 in glucose counterregulation.

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The insulin-like growth factor binding proteins (IGFBP) are a group of structurally homologous proteins that specifically bind insulin-like growth factors (IGF) I and II. Complementary DNA sequences for six rat and human IGFBP have been identified (1–7). By convention, the IGFBP are designated as IGFBP-x, where x represents the temporal sequence of full nucleotide or cDNA publication; and the IGFBP are labeled with arabic numerals, whereas the IGF are labeled using roman numerals (8). Each of the six IGFBP has specific tissue production and distribution sites, leading to the hypothesis that this group of proteins evolved to regulate tissue-specific actions of the IGF.

Most of the circulating plasma IGF-I and IGF-II is associated with IGFBP-3 and an acid-labile subunit in a growth hormone-dependent tertiary complex which may serve as a relatively static and usually saturated

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IGF reservoir (9, 10). IGFBP-3 is present in mg/liter quantities in normal human serum. IGFBP-2 is also present in normal serum in relatively static mg/liter quantities (about 10-fold lower than IGFBP-3), and only limited information is available regarding its regulation and function (11–15). Even less information is available regarding IGFBP-4, IGFBP-5, and IGFBP-6, all of which are present in relatively low quantity in plasma (6, 7, 16–18).

IGFBP-1 is the only one of the six IGFBP which appears to have rapid dynamic regulation in human plasma, with levels that may normally vary more than 10-fold within a few hours (1, 19, 20). The molecular characteristics of IGFBP-1 are consistent with rapid regulation of IGFBP-1 mRNA production and protein clearance, and both *in vivo* and *in vitro* investigations indicate that plasma IGFBP-1 fluctuations are largely due to insulin regulation of IGFBP-1 transcription (19, 20). In this paper, we review data relating to the structure, synthesis, regulation, and function of IGFBP-1.

Historical Perspective

The history of IGF and IGFBP investigations has been reviewed recently and only salient points are repeated here (1–5, 21–24).

In 1957, Salmon and Daughaday (25) reported a serum factor that mediated the cartilage sulfation activity of growth hormone (GH). A few years later, other investigators described a serum insulin-like activity that was not suppressed by anti-insulin antibodies (26–28). Studies in the mid- to late 1960s demonstrated that both serum sulfation factor and serum nonsuppressible insulin-like activity are primarily present in a large molecular mass complex with an estimated molecular mass of >100 kDa (29). Acid treatment of this complex released an active 5- to 10-kDa peptide. In 1972, these small molecular mass peptides were designated somatomedins, replacing the nonsuppressible insulin-like activity and sulfation factor terminology (30). Shortly thereafter, two mammalian somatomedins were identified by protein sequence and cDNA data (31–34), and their structural homology with proinsulin led to their current designation as IGF-I and IGF-II (35).

In the mid-1970s, studies by Hintz and Liu (36–38) and others (39–42) showed that the molecular mass distribution of the somatomedins in human serum is due to the presence of serum IGF-binding activity. By size-exclusion gel filtration chromatography in neutral pH buffer, most of the serum IGF was present in a saturated ~150 kDa complex. This complex was dissociated by acidification, leading to the appearance of free IGF peptide at ~5–10 kDa and increased amounts of a ~30–50 kDa unsaturated IGF-binding activity (38, 42, 43). Similar data were reported in rat serum (44) and IGFBP activity was identified in several body fluids as well as in conditioned medium from different cell lines (4, 45). These observations led to several proposed models for the IGF/IGF-binding protein phenomenon (22, 37, 46). However, definitive studies were hindered by a lack of purified IGFBP and technical limitations.

In the mid-1980s, the application of new techniques in protein chemistry and molecular biology greatly facilitated IGFBP investigations. Essentially identical N-terminal sequences were reported for a ~30-kDa IGFBP isolated from human amniotic fluid and HepG2 human hepatoma cell-conditioned medium (47–51), apparently representing the ~30-kDa serum-unsaturated IGFBP. Various studies gave slightly different molecular mass estimates, leading to a diverse nomenclature (IGFBP26, IGFBP25, IGFBP28, etc.) complicated by an alternate nomenclature based on source and physiology (amniotic fluid IGFBP, HepG2 IGFBP, GH-independent IGFBP) (8).

In unrelated investigations, Bohn and co-workers (52, 53) had identified several placental tissue antigens that were sequentially numbered. One of these antigens, placental protein 12 (PP12), originally reported in 1980 (52), was found in 1986 to have IGF-binding activity and an N-terminal sequence identical to that of amniotic fluid and HepG2 cell-derived IGFBP (54). Another protein subsequently identified as IGFBP-1 is pregnancy-associated endometrial α_1-globulin, originally characterized as the major protein secreted by decidualized endometrium during human and baboon pregnancy (55, 56).

Specific antibody preparations and radioimmunoassays for PP12 and many of the other proteins mentioned were developed that facilitated molecular studies (57–61). A major technical advance was the development of the Western ligand blot reported by Hossenlop *et al.* (62) (see Fig. 2 for example).

In 1988, we reported the cDNA sequence for the HepG2-derived IGFBP (63). Identical sequences were subsequently reported from human placental and decidual cDNA libraries (64–66). A cDNA sequence from a human decidual library reported by Brewer *et al.* (67) differed from other reported sequences between residues 30 and 46. A subsequent communication corrected this sequence, resulting in identity with other reported IGFBP-1 cDNA sequences (68). The protein predicted from the cDNA sequence described from our laboratory and others is now designated IGFBP-1 (8).

Molecular Characterization of IGFBP-1

IGFBP-1 Protein. The biochemical characteristics of IGFBP-1 (PP12) were first reported by Bohn and Kraus (52) in 1980. By ultracentrifugation, a sedimentation coefficient of 2.7S and a molecular mass of 25.2 kDa were calculated, and were virtually identical to the molecular mass later predicted from cDNA and protein sequence data. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of the purified protein, however,

revealed a molecular mass of 51 kDa, which, in retrospect, may have been due to dimerization. An isoelectric point of 4.6–4.7 and significant levels of glycosylation (4.3% carbohydrate) were also reported.

The complete amino acid structure of human IGFBP-1 has been predicted from the cDNA sequence and confirmed by direct sequencing of purified IGFBP-1 protein (63–66). Human IGFBP-1 consists of 234 amino acids and has a predicted molecular mass of 25.3 kDa. A protein polymorphism has been demonstrated, with Met-228 predicted by some cDNA and Ile-228 by others (66); this difference is unlikely to be of functional significance.

Hydropathic analysis of the primary amino acid sequence shows that the 25-residue N-terminal signal peptide and the cysteine-rich N terminus are extremely hydrophobic, while the remaining areas of the molecule are hydrophilic (63). This correlates with the low percentage of charged amino acids, 10%, in the hydrophobic N terminus, as compared with 27% in the hydrophilic C terminus.

The primary amino acid sequence of human IGFBP-1 is remarkable for an N-terminal cluster of 12 cysteines and a C-terminal cluster of six cysteines. These same 18 cysteines and their two-dimensional spatial orientation are conserved in rat and bovine IGFBP-1 and in human and rat IGFBP-2 through -5. Cysteines 3 and 4 of IGFBP-1 through-5 are not found in human or rat IGFBP-6 and cysteines 6 and 7 are not found in rat IGFBP-6 (6, 7, 11, 16, 17, 69–74). All 18 cysteines of IGFBP-1 appear to be involved in intrachain disulfide linkages (75), which may provide a structural framework, with surrounding conserved residues providing functional specificity for IGF-binding and other actions (63, 75).

The IGFBP-1 protein can be divided into three regions according to structural characteristics (Fig. 1). Region 1 contains the first 79 residues of human IGFBP-1 including the N-terminal cysteine cluster; sixty-five of these 79 residues (82%) are conserved in bovine and rat IGFBP-1 and 23 of 79 (29%) are conserved in the other five human IGFBP. Residues 59–79 of human IGFBP-1 are 43% similar with residues 133–153 of the precursor for human transforming growth factor-α; the functional significance of this weak similarity is not clear. These same residues are conserved in rat and bovine IGFBP-1, but not in the other human IGFBP.

Residues 30–53 of human IGFBP-1 form a hydrophobic domain which is conserved in the rat and bovine forms and which has been hypothesized to participate in ligand binding (63). The notion that the N-terminal region of IGFBP-1 participates in ligand binding is supported by the following data: (i) a 21-kDa N-terminal IGFBP-1 fragment retains the ability to bind IGF peptides (76), (ii) deletion of the 60 N-terminal residues of IGFBP-1 abolishes IGF binding (75), and (iii) site-directed mutagenesis of five N-terminal Cys residues (Cys-5, -8, -16, -32, and -34) to Ser residues results in expression of appropriately sized IGFBP-1 proteins that are recognized by IGFBP-1 antiserum but show greatly decreased (Ser-8 and -34) or absent (Ser-5, -16, and -32) IGF-I binding by ligand blot (Fig. 2).

Noncysteine N-terminal residues that might be involved in IGF-binding have not yet been identified. Brinkman *et al.* (75) studied eight IGFBP-1 proteins containing point mutations in the N-terminal region and only one (Cys-38 to Tyr-38) exhibited loss of IGF-binding activity, suggesting that the IGF-binding domain may be diffuse and not limited to the N terminus. However, only one of the remaining seven mutants contained an altered residue that is conserved in all IGFBP; mutations of other conserved residues might reveal other N-terminal residues that are essential for IGF binding.

Region 2 of human IGFBP-1 spans residues 80–144. Only 26 of these 65 residues (40%) are conserved in rat and bovine IGFBP-1, and homology with IGFBP-2 through -6 does not exist in this region. IGFBP-1 is rich in Pro, Glu, Ser, and Thr. Residues 89–114 of region 2 form a typical Pro-Glu-Ser-Thr (PEST) domain (65), which includes clusters of these four amino acids flanked on each side by a positively charged amino acid. PEST domains are also present in Region 2 of rat and bovine IGFBP-1. The presence of PEST regions coupled with the negative charge of the IGFBP-1 protein suggests that IGFBP-1 is rapidly metabolized. Other proteins with similar PEST regions and rapid turnover rates include c-*fos*, c-*myc*, and ornithine decarboxylase (80).

Region 3 of human IGFBP-1 spans residues 145–234 and includes the C-terminal cysteine cluster. Sixty-one of these 90 residues (68%) are conserved in rat and bovine IGFBP-1 and 13 of 90 (14%), including the six cysteines, are conserved in the five other human IGFBP. The two-dimensional spatial orientation of the cysteines is conserved not only among the six IGFBP, but also in 10 N-terminal repeats of the thyroglobulin molecule and with similar domains found in a gastrointestinal tumor antigen and in the invariant chain of the class II major histocompatibility antigen (16). Residues 151–165 of human IGFBP-1 are 47% similar with residues 4–18 of human retinol-binding protein (65); this similarity is unlikely to be significant since it is not observed in rat or bovine IGFBP-1 or in any other IGFBP.

The function of Region 3 is unknown but it may participate in IGF binding since the following mutations in this region abolish the ability of IGFBP-1 to bind IGF-I on ligand blot: (i) point mutations at Cys-226 and nearby residues (81), (ii) deletion of the C-terminal 20 residues (81), and (iii) deletion of amino

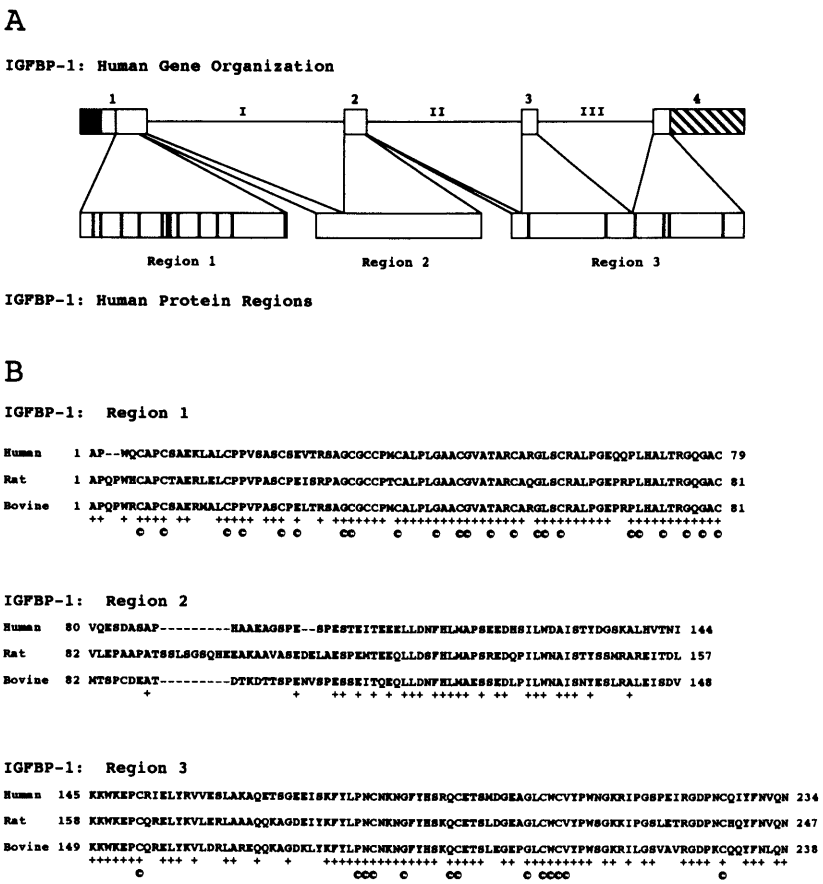

Figure 1. IGFBP-1 gene and protein organization. (A) Schematic representation of the human IGFBP-1 gene (above) and protein (below) organization. The IGFBP-1 gene consists of four exons represented by the boxes labeled 1 through 4, and three introns represented by the solid lines labeled I, II, and II. The solid box represents the 5′-untranslated sequence of Exon 1; the stippled box represents the 25-amino acid signal peptide; the open boxes represent regions of the four exons that encode amino acid sequence for the mature IGFBP-1 protein; the cross-hatched box represents the 3′-untranslated sequence of Exon 4. The IGFBP-1 protein is divided into three regions, and the relationship of these regions to the four exons is indicated by diagonal lines. Protein Region 1 is roughly equivalent to Exon 1, Region 2 is roughly equivalent to Exon 2, and Region 3 is roughly equivalent to Exons 3 and 4. The 18 IGFBP-1 cysteines are represented by solid lines within protein Regions 1 and 3. (B) Comparison of human, rat, and bovine IGFBP-1 amino acid sequences. These sequences are divided into the three protein regions described above for human IGFBP-1. Amino acids conserved among the three IGFBP-1 sequences are indicated by +; amino acids also conserved among the other five human IGFBP are indicated by @. Human, rat, and bovine IGFBP-1 sequences are from Refs. 63, 71, and 72, respectively; human IGFBP-2, -3, -4, -5, and -6 sequences are from Refs. 11, 73, 69, 7, and 6, respectively.

acids 151–226 (d151–226), which results in a small (20 kDa) IGFBP-1 form devoid of the six C-terminal Cys residues but still recognized by IGFBP-1 antiserum (Fig. 2). In addition, a C-terminal fragment of rat IGFBP-2 (39/132 residues shared with rat IGFBP-1 in this region) is able to bind ligand (82). In conjunction with the previously mentioned data relating to the N terminus, the data strongly indicate that both N- and C-terminal regions, and particularly the cysteine residues, contribute determinants required for optimal IGF binding. Confirmation of this hypothesis awaits determination of the three-dimensional structure of IGFBP-1.

An Arg-Gly-Asp (RGD) sequence is located in Region 3 of human IGFBP-1 at residues 221–223, and is also found in rat and bovine IGFBP-1, and in rat and human IGFBP-2 (6, 11, 16, 70–72, 74). RGD sequences are present in a group of extracellular matrix proteins (integrins) and are thought to mediate binding of these proteins to cell membranes (83), leading to the hypothesis that IGFBP-1 itself might function as an extracellular matrix protein. This was supported by unpublished data concerning competitive cell membrane binding of IGFBP-1 and synthetic RGD (67). However, the physiologic significance of the IGFBP-1 RGD sequence is currently undefined.

Posttranslational modification of IGFBP-1 has also been studied. IGFBP-1 does not contain *N*-linked glycosylation sites; *O*-glycosylation at Ser and Thr residues probably accounts for its 4.3% carbohydrate content (52, 65). The functional significance of IGFBP-1 glycosylation has not been defined; however, it may be important for metabolic processing.

Human IGFBP-1 is secreted as a phosphoprotein with phosphorylation limited to serine residues, principally Ser-101 (70%) and, to a lesser extent, Ser-169 (25%) and Ser-119 (5%) (84, 85). Ser-101 and Ser-119

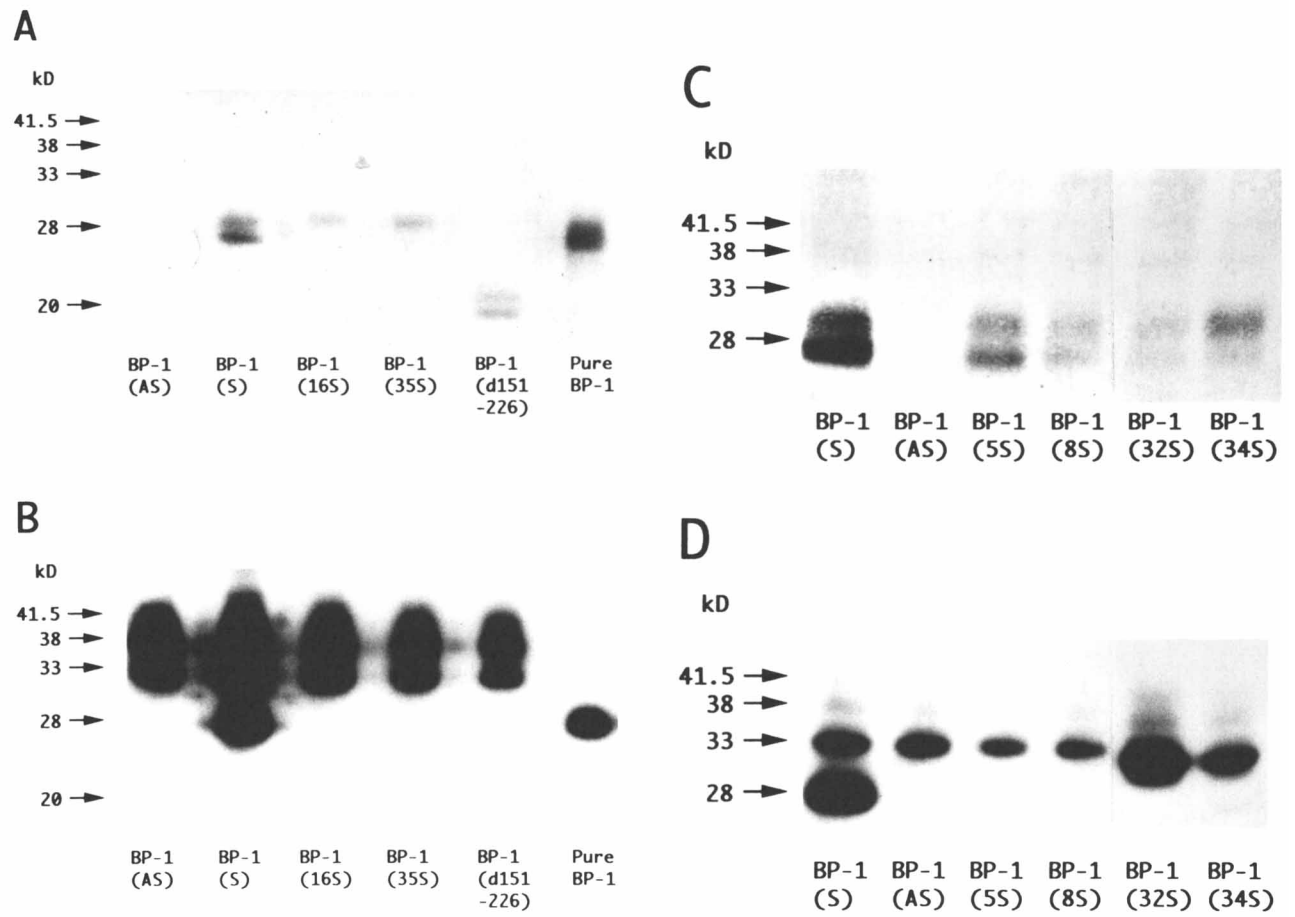

Figure 2. IGF-I binding of native and mutant IGFBP-1 peptides. The human IGFBP-1 cDNA (63) was inserted into M13 and site-directed mutations were created as described previously (77); Cys-5, -8, -16, -32, and -34 residues were mutated to Ser residues (5S, 8S, 16S, 32S, 34S, respectively) using 25 base oligonucleotides with the central TG(T/C) Cys codon replaced by the TC(T/C) Ser codon, while the deletion of residues 151–226 (d151–226) was created with a 24-base oligonucleotide complementary to the four codons on each side of the deleted segment. Native IGFBP-1 cDNA was inserted into the p91205(B) expression vector in sense (BP-1 S) and antisense (BP-1 AS) orientations, while mutant cDNA were inserted in the sense orientation; 30 mcg of each construct was then transfected transiently into 10^6 COS-1 cells by the calcium phosphate method (78). After 3 days, conditioned medium was concentrated 10-fold, separated by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to nitrocellulose. Transferred proteins were analyzed by Western immunoblot (A and C) using a polyclonal antiserum to purified IGFBP-1 (79) and by Western ligand blot (B and D) using ^{125}I[IGF-I] as ligand (62). IGFBP-1 migrates as a 28-kDa protein by both immuno and ligand blot. BP-1(d-151–226) migrates as a 20-kDa protein by immunoblot. The 41-, 38-, and 33-kD IGBP seen on ligand blot are IGFBP-3 and IGFBP-2 forms secreted constitutively by the COS-1 cells.

are conserved in rat and bovine IGFBP-1, but Ser-169 is not. Phosphorylation appears to be associated with increased affinity for IGF peptides. Site-directed mutagenesis of Ser-101 to Ala-101 prevents phosphorylation of this residue and results in a 3-fold decreased affinity for IGF-I (85).

A phylogenetic tree for the six human IGFBP demonstrates that IGFBP-1 is most closely related to IGFBP-4 and IGFBP-2, while the other IGFBP show early divergence from this group (Fig. 3) (86, 87). Interspecies evolutionary analysis of IGFBP-1 itself is not feasible since protein and cDNA sequences are available only for a few mammalian species. IGFBP-1 has not been reported in nonmammalian species.

IGFBP-1 mRNA. Human IGFBP-1 cDNA from placenta, uterine decidua, liver, and hepatoma cell-derived libraries are essentially identical (63–68). Primer extension studies using RNA from HepG2 human hepatoma and uterine decidual cells identify the IGFBP-1 transcription start site at 165 bp upstream from the ATG translation site (88, 89). Thus, human IGFBP-1 mRNA is a transcript of ~1.55 kb consisting of 165 bp of 5′-untranslated sequence, 777 bp of coding sequence, and at least 612 bp of 3′-untranslated sequence. The 3′-untranslated region contains five ATTTA motifs that are characteristic of mRNA species with a very short half-life (90). The presence of PEST regions in the IGFBP-1 protein and the ATTTA motifs in the IGFBP-1 mRNA may explain the rapid and marked fluctuation of IGFBP-1 levels *in vivo*, a phenomenon that is discussed in a later section.

Human IGFBP-1 cDNA have been used to perform Northern blots and RNase protection assays in a number of human tissues and cell lines (63–67). These

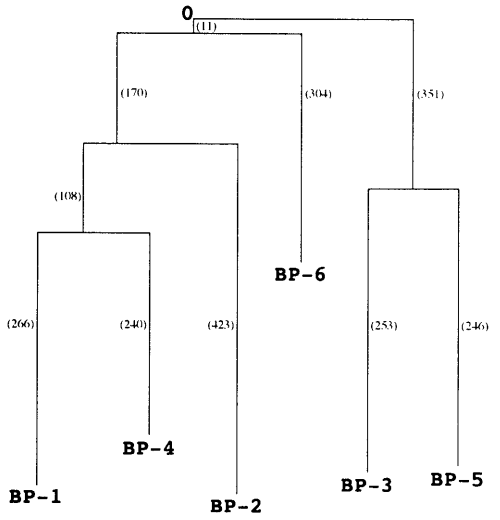

Figure 3. Phylogenetic tree for the six human IGFBPs. A phylogenetic tree was constructed after the method of Feng and Doolittle (86). References for the amino acid sequences are listed in the legend for Figure 1. *O* indicates the origin. Branch lengths (numbers in parentheses) are calculated to be proportional to true evolutionary distances.

studies demonstrate the tissue-specific expression of a single ~1.55-kb IGFBP-1 mRNA transcript, consistent with the size predicted by cDNA sequencing and primer extension studies. The human IGFBP-1 transcript is expressed in normal fetal liver, and in postnatal tissues primarily in secretory endometrium, pregnancy decidua, and liver. Low levels of expression have been found in Wilm's tumor, but not in normal human fetal or postnatal kidney. IGFBP-1 mRNA and protein expression has also been identified in an ovarian cystadenoma, in primary isolates and cultures of human ovarian granulosa-luteal and decidual cells, and in several tumor-derived cell lines, including HepG2 human hepatoma, HEC1B and KLE endometrial adenocarcinoma, and MDA-MB-231 and Hs578t breast cancer cell lines (91–99). IGFBP-1 expression is not detected in normal or cancerous breast tissue or in eight other breast tumor-derived cell lines (98); thus, a role of IGFBP-1 in the pathogenesis of breast cancer is uncertain.

In the rat, IGFBP-1 mRNA is also found as a single 1.5-kb transcript with four ATTTA motifs in the 3′-untranslated region (70, 71). In contrast to the human, rat kidney expresses easily detectable amounts of IGFBP-1 (70, 71, 100); other mRNA sites are similar between species. In monkeys, the major sites for IGFBP-1 mRNA expression are similar to those in humans (101, 102), with the possible exception that IGFBP-1 mRNA is also expressed in rhesus monkey kidney (F. Liu, D. R. Powell, and R. L. Hintz, unpublished observations).

IGFBP-1 Chromosomal DNA. The human IGFBP-1 chromosomal gene has been isolated and completely sequenced (87, 88, 103, 104). The gene is divided into four exons and spans 5.2 kb of chromosomal DNA (Fig. 1). Exon 1 spans 514 bp and includes the entire 5′-untranslated region. This is followed by sequence encoding the first 91 amino acids, roughly corresponding to Region 1 of the IGFBP-1 protein as described above. Exon 2 spans 170 bp, which encodes most of the amino acid sequence found in Region 2. Exon 3 spans 129 bp and encodes approximately the first half of the IGFBP-1 Region 3 sequence, while the 701 bp of exon 4 encodes the second half of Region 3 and the entire 3′-untranslated sequence of IGFBP-1 mRNA.

The exon/intron structural organization of human IGFBP-1 is remarkably similar to human IGFBP-2, -3, and -5, except that IGFBP-3 has a fifth exon that contains sequence found only in the 3′-untranslated region of the mRNA (88, 105, 106). As expected based on the IGFBP-1 protein regions encoded by each exon, exons 1, 3, and 4 are homologous among these four IGFBP genes, but exon 2, which encodes protein Region 2, is not homologous.

The size of genomic DNA fragments that hybridize with IGFBP-1 cDNA probes was compared with the size of restriction fragments predicted from the IGFBP-1 gene sequence (65, 67). This analysis strongly suggests the presence of a single IGFBP-1 gene in the human genome (88). That (i) a single 1.55-kb IGFBP-1 mRNA species has been identified in all human tissues expressing this protein, (ii) all human IGFBP-1 cDNA characterized to date exhibit only minor sequence differences, and (iii) IGFBP-1 transcripts from human liver and decidua initiate at the same mRNA cap site also suggest the presence of a single IGFBP-1 chromosomal gene that is not differentially spliced to any appreciable extent. Even the minor sequence differences identified in the reported IGFBP-1 cDNA can be explained by the presence of two allelic variants at the same gene locus which account for the established protein polymorphism at residue 228. Thus Allele 1, which was detected in four of 18 unrelated Scandinavians, encodes Met-228, while Allele 2, which was present in the remaining 14 individuals, encodes Ile-228 (104).

The IGFBP-1 gene has been localized to human chromosomal region 7p14-p12 (107, 108). The gene for IGFBP-3 is also localized to chromosomal region 7p14-p12, while genes for the other four IGFBP are reportedly located on other chromosomes (6, 7, 12, 104, 105). Detailed analysis reveals that the IGFBP-1 and -3 genes are contiguous, arranged in a tail-to-tail fashion separated by only 20 kb of chromosomal DNA (104). This spatial arrangement suggests that these IGFBP genes were duplicated by inversion, which is consistent with the evolution of their significant sequence differences and functional diversity. At present, there is no evidence that these genes share regulatory elements in their intergenic region. Comparison of individual hu-

man and rat IGFBP sequences demonstrates at least 67% amino acid identity, indicating that rearrangements leading to evolution of different IGFBP forms occurred before the mammalian evolutionary radiation. Thus, the IGFBP-1 and IGFBP-3 genes are likely to be chromosomally linked in other mammalian species.

Interestingly, members of the homeobox (HOX) gene family, which regulate spatial organization in the developing fetus, are located in the same chromosomal regions as the IGFBP genes. IGFBP-1 and IGFBP-3 are found in the same region as HOX1, IGFBP-2 is close to the HOX4(A-F) genes, and IGFBP-4 is located in the same region as HOX2(A-I) (105). This implies past duplication of an entire chromosomal segment containing ancestral IGFBP and HOX genes, and suggests that these IGFBP and HOX genes may be linked in other mammalian species.

The region 5′ to the human IGFBP mRNA cap site functions as a gene promoter (77, 87). Beginning 28 bp 5′ to the cap site is a TATA element, important for accurate initiation of transcription in genes transcribed by RNA polymerase II. In addition, a CCAAT upstream promoter element is located 72–68 bp 5′ to the cap site. The cap site and exon 1 are located in a CpG island, typical of eukaryocytic promoters (109).

The first 1205 bp 5′ to the cap site can direct efficient expression of the reporter genes chloramphenicol acetyltransferase and luciferase (77, 87, 110). In the case of chloramphenicol acetyltransferase expression, the chloramphenicol acetyltransferase mRNA transcribed under the direction of the putative IGFBP-1 promoter is initiated at the established IGFBP-1 cap site, strongly suggesting that the IGFBP-1 promoter is located in this 1205-bp region (77). Recent studies demonstrate a similar location for the rat IGFBP-1 mRNA cap site and show that the first 470 bp 5′ to this cap site are highly conserved in the human sequence (111). These observations suggest that many of the elements which play an important role in IGFBP-1 gene transcription are located within this 470-bp region. Although it is likely that elements outside of this region interact with this regulatory sequence, there is currently no evidence for additional promoter regions that can independently regulate IGFBP-1 transcription.

Plasma Levels of IGFBP-1

The first quantitative measurements of IGFBP-1 were radioimmunoassays for PP12, now known to be IGFBP-1. PP12 is present in ng/ml quantities in normal serum (52, 53), with lower levels in plasma, implying proteolysis (52). Levels are elevated in pregnant maternal serum, with peak levels in midgestation (112, 113). Amniotic fluid levels are 100–1000 times higher than in maternal serum (53, 59). Significant diurnal variation of PP12 levels were shown in pregnant women, with the highest levels in the morning (113). Pathologic conditions associated with elevated PP12 levels include hepatic cirrhosis and carcinoma (114), some cases of trophoblastic disease (112, 115), and pregnancies complicated by twin gestation, pre-eclampsia, or maternal diabetes (52, 116). Immunohistochemical and immunoassay studies of female reproductive structures show that PP12 protein is limited to the syncytiotrophoblast, placental decidua, secretory phase endometrium, and luteal phase ovarian granulosa cells. PP12 is also found in fetal, but not adult, human liver (53).

Drop *et al.* (117, 118) developed a radioimmunoassay using a partially purified preparation of human amniotic fluid IGFBP-1 and reported that serum levels are elevated in fetal life, and decline postnatally. Elevated maternal serum levels during pregnancy were also confirmed. Absolute quantitition was not possible due to the lack of purified standard.

Baxter *et al.* (119) also reported a midgestational IGFBP-1 peak in amniotic fluid, and IGFBP-1 levels were inversely correlated with the lecithin to sphingomyelin ratio, a marker of fetal maturity.

We have also found high levels of IGFBP-1 in fetal serum (120) (Table I). Fetal serum levels appear to rise in midgestation, coincident with the timing of fetal hepatic maturation. Fetal serum levels are approximately 10- to 20-fold higher than reported maternal serum levels, which also rise in midgestation (53, 112, 121). On the other hand, fetal levels are approximately 5- to 10-fold lower than amniotic fluid levels at each stage of gestation (53, 119, 122). As demonstrated by both size-exclusion gel chromatography and protein immunoblot studies, these elevated fetal and amniotic fluid levels are not due to increased degradation products (120). The source and function of fetal IGFBP-1 are not defined, and the relative contributions of fetal and maternal production to amniotic fluid, fetal, and maternal levels are not known. However, given that growth hormone has a limited role in fetal growth and IGF production (123), it is interesting to speculate that IGFBP-1 may play a critical role in controlling the mitogenic and metabolic effects of the IGF in fetal life. In support of this hypothesis are the findings that cord blood IGFBP-1 levels are decreased and inversely correlated with birthweight in infants of diabetic mothers and are decreased in large part for gestational age infants (113, 124–126). Moreover, fetal serum IGFBP-1 levels are elevated in intrauterine growth-retarded rats and humans (124, 127). An inverse relationship of fetal cord blood IGFBP-1 and insulin levels has also been reported (124, 128).

Fetal, amniotic fluid, and maternal IGFBP-1 levels decline in late gestation, and fall precipitously at parturition in both maternal and fetal serum (53, 119, 120). Levels then show a progressive age-related decline in normal, healthy individuals (57, 118, 129). Unlike

Table I. IGFBP-1 Levels in Normal Human Body Fluids

Fluid	Sample timing	Comments, *n* [a]	IGFBP-1[b] (mcg/liter)	Ref.
Amniotic fluid	14–20 wk	48	51,000	126
	15 wk	54	23,013 ± 8,858	112
	20–22 wk	40	51,000	59
	3rd trimester	40	37,600 ± 17,600	119
	36–40 wk	7	15,300 ± 7,875	112
Fetal[c]	Urine	3	93 (23–139)	*,120
	Serum, 107–110 wk	5	1,903 (369–4,612)	"
	Serum, 112–120 wk	8	2,945 (178–9,105)	"
	Serum, 122–130 wk	8	4,399 (96–8,173)	"
	Serum, 131–140 wk	4	2,545 (619–4,483)	"
	Serum, 143-term	8	1,518 (187–5,488)	"
	Cord serum		43 ± 22	112
	Cord serum	23	56.9 ± 34.6	145
	Cord serum	14	91 (34–304)	125
	Cord serum	14	191 (55–1,698)	59
Pregnant maternal[d]	22–23 wk	353	169 ± 126	112
	27 wk	420	102.8 ± 38.0	116
	32–33 wk	353	63 ± 23	112
	Term	353	48 ± 25	"
	Term	420	121.3 ± 46.0	116
	Term	14	113 (58–281)	125
Milk	Postpartum Day 1	41	165 ± 80	182
	Concurrent maternal serum, Day 1		136 ± 68	"
	Postpartum Day 3	41	97 ± 70	182
	Concurrent maternal serum, Day 3		158 ± 57	"
Children	Basal	15	17.4 ± 3.2 (SE)	152
	Fasting	15	219 ± 30 (SE)	"
	Fasting	21	109 ± 5	153
	Fasting, 18 hr	6	239 ± 30	"
Children, by age	Nonfasting	Newborn, 12	2,609 (280–10,341)	*,120
	Fasting	1–10 yr, 24	465 ± (32–1,130)	*,58
	Fasting	Puberty, 6	242 ± 53 (83–473)	*,135
	Nonfasting	0–0.99 yr, 10	69 (21–418)	129
		1–3.99, 28	55 (14–338)	
		4–7.99, 18	33 (12–125)	
		8–9.99, 26	25 (13–65)	
		10–15.5, 67	19 (10–72)	
Puberty	Fasting, male	Tanner 1, 18	112 ± 8	157
		Tanner 2, 5	64 ± 9	
		Tanner 3, 6	59 ± 9	
		Tanner 4, 3	64 ± 5	
		Tanner 5, 8	45 ± 9	
	Fasting female	Tanner 1, 7	86 ± 5	157
		Tanner 2, 3	83 ± 6	
		Tanner 3, 6	54 ± 11	
		Tanner 4, 6	46 ± 5	
		Tanner 5, 9	40 ± 7	

—Table I continues

IGF-I and IGFBP-3, IGFBP-1 levels fall rather than increase during puberty and are inversely correlated with age, pubertal stage (124), IGF-I levels (57, 129), and sex-hormone binding globulin levels (130). No consistent variation of IGFBP-1 levels with the menstrual cycle has been observed (131).

Representative immunoassay levels of IGFBP-1 in normal human body fluids are shown in Table I.

IGF inhibitors have been identified in childhood chronic renal failure and have been postulated to play a role in the associated linear growth retardation. Serum IGF-binding activity is elevated in growth-retarded children with end-stage renal disease, while IGF-I and IGF-II levels are normal (132–134). In this same population, fasting IGFBP-1 levels are 10- to 100-fold higher in children with chronic renal failure as compared with age- and sex-matched controls (58). Furthermore, IGFBP-1 levels were directly correlated with serum

Table I. Continued

Fluid	Sample timing	Comments, n [a]	IGFBP-1[b] (mcg/liter)	Ref.
Adults		7	23 (18–36)	59
	Nonfasting	8	40 (24–81)	*
	Fasting	5	120 ± 12	154
	Fasting	8	72 ± 15 (SE)	161
	Fasting	6	47 ± 9 (SE)	162
	Fasting	6	52 ± 9	147
	Fasting	73	37 ± 2	156
	Fasting	6	89 ± 56	150
	Fasting	15	9.4 ± 4.4	145
	Fasting	73	34 (12–91)	129
Adult male	Nonfasting	34	12.8 ± 3.5	112
	Fasting	11	45 ± 24	149
Adult female	Nonfasting	70	19.0 ± 7.8	112
	Fasting	6	44.9 ± 13.2 (SE)	160
	Fasting	19	41 ± 17	151
	Nonfasting	8, fertile	39 ± 1.8 (SE)	182
	Nonfasting	23, menopausal	36 ± 6.0 (SE)	"

[a] The number shown is the number of data points.
[b] Immunoassay data are presented as the mean ± SD except as noted: SE or range (in parentheses).
[c] Fetal serum and urine samples were obtained from Dr. T. Shepard, Central Laboratory for Human Embryology, University of Washington, Seattle, WA, and were collected from elective abortions. Gestational ages were estimated by dates and fetal crown-rump length. Several fetuses had dysmorphic and chromosomal abnormalities.
[d] Pregnant maternal serum was nonfasting in most cases.
* P. D. K. Lee, unpublished data.

IGF-binding activity as measured by charcoal-separated ligand binding assay, and the elevated levels are not due to IGFBP-1 degradation products (58). These data imply that the excess unsaturated IGF-binding activity in chronic renal failure may, in part, be due to impaired metabolism and/or clearance of intact IGFBP-1. Similar elevations of IGFBP-1 levels have been demonstrated in adolescents and adults with chronic renal failure (135, 136). IGFBP-1 levels have been reported to decrease in children with chronic renal failure treated with GH (137, 138).

Insulin-dependent diabetes mellitus (IDDM) is another condition in which IGFBP-1 appears to be abnormally regulated, with elevated levels reported in several studies (139, 140). In a study of 79 children with IDDM, we found that the normal inverse correlations of serum IGFBP-1 with age, Tanner pubertal stage, and IGF-1 levels were absent (57). In the prepubertal children with IDDM, IGFBP-1 levels were correlated with glycated hemoglobin levels, and inversely correlated with insulin *dose corrected for body weight* (insulin levels were not studied). These correlations were lost in the pubertal group, resulting in relatively high IGFBP-1 levels after puberty.

Other studies of IGFBP-1 plasma levels show low levels in obesity, polycystic ovary syndrome (141–145), and acromegaly (129, 146) and elevated levels during prolonged fasting, prolonged exercise, in intensive care patients, and in anorexia nervosa (129, 146–150). In some of these conditions, fasting IGFBP-1 levels have been demonstrated to be inversely related to insulin levels (142, 143, 146, 151).

Early investigations of human IGFBP-1 plasma levels conducted by Baxter and co-workers (152, 153) described rapid daily fluctuations ranging as much as 10-fold or higher in normal, healthy individuals. A similar phenomenon had been described in earlier studies of PP12 (53). This was initially thought to represent a type of circadian rhythm or diurnal variability, but more detailed study showed that falls in IGFBP-1 levels occurred immediately after a meal, while increases occurred during fasting (154). Similar fluctuations were demonstrated in GH-deficient children and in acromegalic adults, implying that GH has no direct role in this IGFBP-1 variability (153).

At about this time, Suikkari and co-workers (151, 155) and Brismar and associates (156) showed that IGFBP-1 levels were correlated with insulin levels in fasting normal individuals, disorders of insulin secretion (including IDDM and insulinoma), and obesity. Holly *et al.* (157) reported that IGFBP-1 levels are inversely correlated with insulin levels during puberty. Furthermore, in adolescents with IDDM, IGFBP-1 correlated with insulin and not GH levels during a euglycemic clamp (158).

An apparent paradox was reported by Yeoh and Baxter (154), who observed that insulin-induced hypoglycemia was associated with a rise in serum IGFBP-1 that occurred approximately 1–2 hr after the insulin dose. This led to the hypothesis that increased glucose,

rather than insulin, might suppress IGFBP-1 levels. Similar results have been reported in rats (159). However, the hypoglycemia-associated rise in IGFBP-1 may also be explained by a secondary suppression of insulin secretion into the hepatic portal circulation, a mechanism which could also explain the temporal delay in IGFBP-1 response.

Snyder and Clemmons (160) showed that fructose could also suppress IGFBP-1 levels. Since fructose causes a lesser rise in insulin levels as compared with glucose, this suggested that insulin-independent glycolysis might regulate IGFBP-1 levels. However, insulin C-peptide levels did increase significantly during the fructose-induced fall in IGFBP-1 levels.

In Vivo Regulation of IGFBP-1 by Insulin: Human Studies

To examine the relative roles of GH, insulin, and glucose in IGFBP-1 regulation, we conducted a study in which seven normal individuals were administered standard meals during three separate conditions: saline infusion, GH infusion, and glucose infusion (161). The glucose infusion was administered to match glucose excursions during the GH infusion. Insulin levels were allowed to fluctuate normally during all three protocols. As expected, IGFBP-1 levels increased during fasting and decreased rapidly after each meal. IGFBP-1 levels were directly correlated with insulin levels and no independent effect of GH was demonstrated during any of the three infusion protocols. As observed previously by Holly *et al.* (158) and Baxter and Cowell (152), we found that physiologic nocturnal GH peaks had no relationship to IGFBP-1 levels during the saline and glucose infusions. Furthermore, IGFBP-1 kinetics were not influenced by the supraphysiologic GH levels during the GH-infusion protocol. Further analysis suggested that glucose also has no effect independent of insulin. From this study, we postulated a "critical" suppressive peripheral plasma insulin concentration of ~90 pmol/liter, above which IGFBP-1 levels decrease.

Since insulin levels were not controlled in this initial protocol, we then conducted a series of experiments to further define this regulation (162). After an overnight fast, a continuous euglycemic hyperinsulinemic clamp caused a rapid fall in plasma IGFBP-1 with a disappearance rate of 0.24 mcg/liter-min and a disappearance $t_{1/2}$ = 89 min; both estimates compare favorably with estimates from previous studies.

During a euglycemic pancreatic clamp (somatostatin + GH infusion) after an overnight fast, IGFBP-1 levels rose >4-fold over 3 hr (162). This latter experiment was repeated using a low, subsuppressive insulin infusion (to prevent endogenous breakthrough insulin secretion), and a plateau IGFBP-1 level was attained after 5 hr, with a $t_{1/2}$ to steady state of 60–75 min.

With a hypoinsulinemic pancreatic clamp, a sequential stepped increase in glucose concentrations from 5 to 9 mmol/liter (3 hr at each concentration) did not have a suppressive effect on the rate of IGFBP-1 increase (162). This provides further evidence that physiologic glucose excursions have no independent effect on IGFBP-1 levels.

Finally, during a euglycemic pancreatic clamp, sequential incremental doses of insulin were infused at 2-hr intervals to define the critical insulin concentration for IGFBP-1 suppression (162). In six healthy adults, the critical insulin concentration was measured at between 65 and 172 pmol/liter. Using a similar protocol, the critical insulin concentration was estimated at between 46 and 100 pmol/liter in three adults with IDDM. Since endogenous insulin secretion was absent during these protocols, these estimates represent insulin concentrations at the liver, which is the primary site for IGFBP-1 production. This compares with the estimates of 90 pmol/liter peripheral insulin for the critical suppressive level from our previous meal-related study in which endogenous insulin levels were not controlled (161), and 65–360 pmol/liter insulin from a cross-sectional study of obese subjects during a somatostatin clamp (155). The results in IDDM are in agreement with data reported by Batch *et al.* (139), and suggests that abnormal IGFBP-1 levels in this disorder are directly related to insulin.

Previous cross-sectional studies of patients with obesity-associated insulin resistance showed a direct inverse correlation of fasting insulin and IGFBP-1 levels (155, 163). We observed similar results in a group of 32 nondiabetic obese and 17 healthy nonobese women (164). When combined with previous data from nonobese adults, a curvilinear inverse relationship was identified between fasting insulin and IGFBP-1 levels, with a sharp inflection point at 70–90 pmol/liter plasma insulin, above which IGFBP-1 levels show little further decline.

Studies in obesity were extended using sequential saline (90 min), hyperinsulinemic (120 min) and hypoinsulinemic (120 min) euglycemic pancreatic clamps after an overnight fast in eight nonobese, four lower-body obese, and six upper-body obese women (164). Mean plasma IGFBP-1 levels were 33, 10, and 6 mcg/L, respectively, during the saline infusion, and were inversely related to the endogenous insulin levels. During the hyperinsulinemic clamp, mean IGFBP-1 levels fell to 13 mcg/liter in the nonobese group, and showed a small but insignificant fall in the obese groups. During hypoinsulinemia, IGFBP-1 levels increased to 78 mcg/liter in the nonobese individuals and only to ~25 mcg/liter in the obese groups. Therefore, it appears that obese individuals have abnormally low IGFBP-1 levels which are inversely related to insulin levels, and that this basal suppression may be associated with a blunted IGFBP-1 response to hypoinsulinemia. Since total IGF-

I levels are normal in obesity, the low IGFBP-1 levels may effectively augment the metabolic actions of IGF-I and could contribute to the pathogenesis of the obesity by enabling the insulin-like actions of the free IGF fraction.

In summary, the data presented in this section allow the following conclusions:

1. Fasting plasma IGFBP-1 levels are inversely related to fasting plasma insulin levels. Studies of IGFBP-1 levels in nonfasting conditions must account for the dynamic effects of insulin on IGFBP-1.

2. Plasma IGFBP-1 levels are not independently correlated with plasma GH or glucose levels.

3. High insulin levels, such as those seen immediately after a meal, are associated with a rapid decrease in IGFBP-1 levels, whereas low insulin levels are associated with a progressive increase in IGFBP-1 levels. From several studies, it appears that the critical suppressive plasma insulin concentration is ~90 pmol/liter.

4. Since IGFBP-1 appears to inhibit the *in vivo* metabolic actions of IGF-I (*vide infra*), low IGFBP-1 levels associated with hyperinsulinemia could play a pathogenetic role in obesity.

In Vivo Regulation of IGFBP-1 by Other Factors: Human Studies

As compared with insulin, GH, and glucose, other factors that may regulate IGFBP-1 *in vivo* are less well characterized. Limited studies of glucagon, somatostatin, and glucocorticoids have been done, and scant data regarding IGFBP-1 metabolism and clearance are available. As discussed in a later section, *in vivo* animal and *in vitro* data suggest possible roles for progesterone, estrogen, IGF, relaxin, and other factors in regulation of IGFBP-1 production; however, little relevant human *in vivo* data are available.

High-dose glucocorticoid treatment is associated with decreased linear bone growth and elevated levels of incompletely identified IGF-inhibitors (165, 166). Although IGFBP-1 levels are apparently decreased with dexamethasone administration (167) and in Cushing's disease (168), secondary hyperinsulinemia was not controlled in these studies. We have recently studied the effects of hypercortisolemia during a hypoinsulinemic, euglycemic clamp (169). During the control saline infusion, IGFBP-1 levels showed the expected rise during hypoinsulinemia. When infused with cortisol, the same subjects showed a >3-fold increase in integrated IGFBP-1 levels over the 6-hr study period as compared with saline-infused controls. These data provide an *in vivo* correlate to *in vitro* (*vide infra*) data showing a facilitative or stimulatory role for glucocorticoids on IGFBP-1 production and suggest a possible role for IGFBP-1 in glucocorticoid-induced catabolism.

Glucagon has been shown to have *in vitro* stimulatory effects on hepatocyte IGFBP-1 (170); however, *in vivo* studies have produced variable results. We have studied IGFBP-1 levels in eight adults with IDDM and nine healthy adults during a hypoinsulinemic, euglycemic pancreatic clamp in which eu-, hypo-, and hyperglucagonemia were induced by glucagon infusion. There was no consistent observed effect of glucagon levels on IGFBP-1 levels during any time period (171; C. A. Conover and P. D. K. Lee, unpublished data). On the other hand, Hilding *et al.* (172) have reported that a single subcutaneous dose of glucagon administered to 15 fasting, GH-deficient adults resulted in a small, but significant, increase in IGFBP-1 levels after 60 and 90 min (36 mcg/liter at baseline to 54 mcg/liter at 90 min). Glucose and insulin levels were reported to rise within 15 to 30 min after dosing.

Subcutaneous administration of the somatostatin analog, octreotide, is reported to cause an acute increase in plasma IGFBP-1 levels in acromegalic adults independent of changes in plasma insulin concentrations (173). Similar results have reportedly been obtained in normal adults during octreotide administration (174). Additional studies will be needed to further characterize this effect.

Progestin-containing intrauterine devices have been reported to induce IGFBP-1 expression in human uterine endometrium (175). Recent studies indicate that hyperestrogenemia is associated with increased plasma IGFBP-1 levels (176, 177). Tamoxifen, used therapeutically as an antiestrogen, also increases plasma IGFBP-1 levels, possibly through an estrogen agonist effect (178).

Finally, short- and long-term administration of IGF-I has been associated with increased IGFBP-1 levels (150, 179, 180). Such an effect could be mediated by IGF-I suppression of insulin secretion; however, additional studies are needed to clarify these results and further define a mechanism.

IGFBP-1 Distribution and Clearance. The physiologic distribution and clearance of IGFBP-1 has direct bearing on control of plasma IGFBP-1 levels. Although early immunohistochemical studies seemed to show IGFBP-1 protein distributed widely through the extravascular space (181), cross-reactivity of the polyclonal antibody for other IGFBP limits interpretation of this data. Monoclonal antibody studies have shown a more limited distribution (55, 56). Human studies using specific radioimmunoassay have found IGFBP-1 in blood (*vide supra*) and milk (182). IGFBP-1 has also been identified by ligand blot in human lymph (183).

Infusions of radiolabeled IGFBP-1 into isolated rat hearts and into intact rats indicate insulin-stimulated transport of circulating IGFBP-1 across cardiac endothelium into the cardiac muscle, but not the connective tissue (184–186). A relatively small amount (~2–3%) of the infused tracer was evidently transported and

IGFBP-1 apparently undergoes rapid degradation in cardiac muscle (185–187); therefore, the relative physiologic contribution of this phenomenon is unknown.

We found no significant difference in IGFBP-1 levels across a peripheral vascular bed during dynamic insulin-induced suppression of plasma IGFBP-1 levels in humans (161), indicating that the contribution of peripheral tissue uptake to IGFBP-1 clearance may not be substantial. However, the overall contribution of tissue uptake of IGFBP-1 in relation to its clearance from plasma remains undetermined.

The clearance rate of IGFBP-1 from plasma is postulated to be extremely rapid, and this supposition is supported by the presence of a PEST domain as discussed in a preceding section. When infused into rats, human IGFBP-1 is cleared with a $t_{1/2}$ of ~12 min, and this is not influenced by simultaneous infusion of IGF-I (188). Similar results have been reported by Young *et al.* (189), who estimated a $t_{1/2}$ for infused radioiodinated human IGFBP-1 of ~7.5 min in rats.

The primary site(s) of IGFBP-1 metabolism and clearance is not known. Components of plasma that are not present in clotted serum appear to cause significant degradation of IGFBP-1 at room temperature, and in plasma samples which are frequently freeze-thawed (113; P. D. K. Lee, unpublished observations). Although the role of circulating proteases in IGFBP-1 degradation remains undefined (190), recent studies demonstrate that plasmin has the ability to completely degrade IGFBP-1 *in vitro* (191).

Degradation products of IGFBP-1 have not been identified in human plasma or serum, although we have observed immunoreactive 14- to 17-kDa fragments in stored HepG2 cell-conditioned medium (P. D. K. Lee, unpublished observations). IGFBP-1 immunoreactivity has been reported in human urine from individuals with mild renal disease (192), but it is not known whether this represents renal production or clearance. As discussed previously, elevated intact IGFBP-1 levels in chronic renal failure (58) suggests a role for renal clearance; however, it is also possible that other metabolic abnormalities in renal failure may inhibit IGFBP-1 degradation and clearance.

In summary, it appears that insulin is the primary physiologic regulator of IGFBP-1 levels in plasma. In the presence of a high endogenous clearance rate, insulin suppression of IGFBP-1 production would be sufficient to account for the rapid and dramatic fluctuations that occur in relation to meals. Little data exist to support a major role for other factors, although high levels of cortisol in the presence of hypoinsulinemia appear to increase IGFBP-1 levels. Glucagon and somatostatin have also been found to increase IGFBP-1 levels in some studies.

Regulation of IGFBP-1: Animal and *In Vitro* Studies

Studies using animal models and cells in culture have shed light on the effect of various physiologic, pathologic, and hormonal states on the regulation of IGFBP-1 expression in various tissues. In most instances, IGFBP-1 regulation mirrors that seen in human *in vivo* studies and yields greater insight into the mechanisms by which IGFBP-1 expression is regulated.

Developmental Regulation. In rhesus monkeys, serum IGFBP-1 levels estimated by ^{125}I[IGF-I] ligand blot were highest in the neonatal period and fell steadily with age, resulting in low levels during adulthood (102). These data were consistent with the hepatic levels of IGFBP-1 mRNA, which were high in fetal and neonatal samples and almost undetectable in pubertal and adult monkey liver. Similar developmental regulation of hepatic IGFBP-1 mRNA expression has been demonstrated in the rat (70, 71, 193). Such observations suggest that serum IGFBP-1 levels are regulated at the level of hepatic mRNA abundance.

Tissue-Specific Expression. As stated previously, IGFBP-1 mRNA is expressed primarily in liver, uterine endometrium, and kidney. Insight into factors regulating this tissue-specific pattern have come from studies characterizing the promoter region of the human gene for IGFBP-1 (77, 87, 88, 103). These indicate that the element crucial to basal IGFBP-1 promoter activity is located between 103 and 61 bp 5′ to the IGFBP-1 mRNA cap site. DNase I protection and gel shift assays using native and mutated IGFBP-1 promoter constructs strongly suggest that hepatic nuclear factor 1 (HNF1, a.k.a. liver factor-B1) is the factor that directs basal promoter activity by binding to a *cis* element spanning from −81 to −53 bp of the IGFBP-1 promoter (77). Recent studies confirm this impression; expression of HNF1 in cells that normally lack this protein results in both binding of HNF1 to the −81 to −53 bp *cis* element and activation of the IGFBP-1 promoter (194). The significance of these findings relates to the fact that HNF1 and the homologous protein, variant HNF1, appear to play important roles in liver development, probably as two of a few master regulatory proteins required to produce the hepatic phenotype. These two HNF1 forms, along with a dimerization cofactor, DCoH, which enhances transcriptional activity of these factors by stabilizing their homodimers, are expressed not only in epithelial cells of liver but also in those of kidney (195–200). Thus, these three proteins, which appear to play a role in developing the phenotype of specialized epithelia, are quite likely to contribute to IGFBP-1 expression not only in hepatocytes, but also in renal tubular epithelial cells (100, *vide infra*). The role of HNF1-related proteins in regulating expression of IGFBP-1 and other genes in endometrial tissues has yet to be explored.

Effect of Partial Hepatectomy. In a search for factors involved in liver regeneration, Mohn *et al.* (70) determined that IGFBP-1 is one of the most highly expressed genes in rat liver after partial hepatectomy. IGFBP-1 mRNA levels in liver increase within 30 min of surgery, peak 250-fold over baseline at 60 min, and are still increased 8 hr postoperatively. In addition, hepatic mRNA levels were found to be superinduced if intraperitoneal cycloheximide was administered at the time of partial hepatectomy, suggesting that the increase in IGFBP-1 mRNA did not require new protein synthesis and that IGFBP-1 is a true immediate early response gene during liver regeneration. The increase in mRNA levels results from a rapid increase in the rate of IGFBP-1 gene transcription, which is elevated several hundred-fold within 30 min of partial hepatectomy and returns to baseline within 6 hr.

Effects of GH and Glucocorticoids. In rats, both GH deficiency and glucocorticoid administration appear to increase hepatic IGFBP-1 expression. Although initial studies found that hypophysectomy led to only a modest increase in hepatic mRNA levels (193), later studies showed 6- and 18-fold increases in hepatic IGFBP-1 mRNA levels in hypophysectomized young and old rats, respectively (201). These changes were associated with a 4-fold increase in the rate of hepatic IGFBP-1 gene transcription. Both short- and long-term therapy with GH led to a marked fall in hepatic IGFBP-1 mRNA levels. The effect of GH was rapid and appeared to be mediated at the level of transcription. A single dose of GH returned hepatic IGFBP-1 mRNA levels and gene transcription rates back to basal levels within 60 min and 30 min, respectively (201). However, the effect of GH on serum IGFBP-1 levels in these rats was not clear, and the possibility that the GH effects might be mediated by other hormones, such as insulin or IGF, must be considered. GH was unable to significantly inhibit IGFBP-1 expression in either primary rat hepatocytes (202) or HepG2 human hepatoma cells (D. R. Powell and A. Suwanichkul, unpublished observations). However, in both cell systems, insulin decreased IGFBP-1 mRNA levels and the levels of IGFBP-1 protein in conditioned medium. In HepG2 cells, this effect of insulin was at the level of transcription (110), suggesting that the effect of GH *in vivo* may be mediated by GH-induced hyperinsulinemia.

Luo *et al.* (203) have shown that administration of dexamethasone to rats resulted in elevated levels of serum IGFBP-1 and a 2- to 10-fold increase in hepatic IGFBP-1 mRNA abundance. The IGFBP-1 transcription rate was not increased 1 hr after dexamethasone injection; other time points and dosing schedules were not studied. Increased IGFBP-1 levels have also been reported in growth-retarded fetal rats after dexamethasone administration (204).

Lewitt and Baxter (170) obtained opposite results in human fetal liver explants, in which dexamethasone suppressed IGFBP-1 release into the medium. However, multiple other *in vitro* studies using H4IIE rat hepatoma cells (205–207) and primary adult rat hepatocytes (202) have found that dexamethasone (0.001–1.0 μmol/liter) significantly stimulates IGFBP-1 protein and mRNA accumulation. In human HepG2 hepatoma cells, dexamethasone stimulates IGFBP-1 promoter activity and augments the stimulatory effects of cAMP and theophylline on mRNA and protein levels (208). In H4IIE cells, the stimulatory effect of dexamethasone occurs primarily at the level of transcription, with increased mRNA stability providing a minor contribution (205, 206). Recent studies in HepG2 cells suggest that the transcriptional effect of dexamethasone is mediated through the proximal IGFBP-1 promoter by *cis* elements located between 357 and 103 bp 5′ to the mRNA cap site (208). Taken together, these studies suggest that dexamethasone is likely to increase IGFBP-1 expression by activating IGFBP-1 transcription.

Effects of Glucagon, Theophylline, cAMP. Lewitt and Baxter (170) found that theophylline, cAMP, and glucagon each stimulated the accumulation of IGFBP-1 in medium conditioned by human fetal liver explants. Later studies using cultured cells confirm the ability of cAMP and theophylline to rapidly stimulate accumulation of IGFBP-1 protein and mRNA (110, 208, 209). In HepG2 cells, the effects of cAMP appear to be at the level of IGFBP-1 transcription (110, 208, 209), and are mediated in part by a cAMP response element (CRE) located at −293 to −249 bp which is capable of binding CRE-binding protein (209).

Since many of the metabolic actions of glucagon are mediated through stimulation of cAMP, it seems reasonable to postulate that glucagon should have a positive effect on IGFBP-1 expression. This effect has been observed in human fetal liver explants (170) but not in HepG2 cells (110). It is not known whether glucagon receptors are present on HepG2 cells. Additional studies will be needed to address this issue.

Other Factors. We have reported previously that progesterone stimulates HepG2 cell production of IGFBP-1 (210, 211). Similar effects have been reported in uterine endometrial explants (96) and primary cultures (212).

Variations in glucose concentration over a physiologic range have no effect on HepG2 cell production of IGFBP-1 (213; C. A. Conover and P. D. K. Lee, unpublished observations). However, glucose concentration below 6 mmol/liter and above 12 mmol/liter may be inhibitory (213).

Octreotide has been reported to stimulate HepG2 cell IGFBP-1 production with an increase in levels of IGFBP-1 mRNA (214). The mechanism of this effect has not been defined.

Protein Kinase C Pathway. Limited studies show

that the phorbol ester, phorbol 12-myristate 13-acetate (PMA), stimulates IGFBP-1 production, indicating that the protein kinase C (PKC) system may be involved in regulation of IGFBP-1 production. This effect was first reported in human ovarian granulosa cells (215).

Phorbol esters also increase IGFBP-1 protein and mRNA levels in rat H4IIE hepatoma cells (216). Significant increases in conditioned medium IGFBP-1 levels were noted within 4 hr of exposure to phorbol-12,13-dibutyrate. This effect was found to be dominant over the inhibitory effects of insulin. Phorbol ester stimulation of IGFBP-1 has also reported in endometrial carcinoma cells (217).

We have conducted detailed investigations of this phenomenon in HepG2 cells (218). PMA stimulates HepG2 IGFBP-1 production in a time- and dose-dependent manner. Maximal stimulation was found at 10–100 mmol/liter PMA, with cell toxicity observed at higher doses. Significant stimulation of IGFBP-1 synthesis was observed within 4 hr of exposure by radioimmunoassay and within 1 hr of exposure by metabolic labeling and immunoprecipitation, with parallel increases in IGFBP-1 mRNA levels (P. D. K. Lee and M. B. Snuggs, unpublished observations). IGFBP-1 levels in 14-hr conditioned medium increased 2-fold over control in confluent and 10-fold in sparse cultures, and levels continued to increase for at least 72 hr after exposure (P. D. K. Lee and M. B. Snuggs, unpublished observations). The effects of PMA are dominant over the inhibitory effects of insulin in this system.

Since PMA is often described as a PKC activator, these results initially suggested that PKC activation stimulates IGFBP-1 synthesis. However, 1,2-dioctanoyl-*sn*-glycerol, a transient PKC activator, had no effect on IGFBP-1 levels (218). Conversely, staurosporine, a PKC inhibitor, stimulates IGFBP-1 production and augments the effects of PMA. Finally, concanavalin A, which inhibits PMA-induced membrane translocation and downregulation of PKC, also inhibited PMA-stimulated IGFBP-1 production. These data have led us to hypothesize that the observed effects of PMA on IGFBP-1 production are due to its action to rapidly downregulate cytosolic PKC after initial activation (218). Therefore, downregulation or inhibition of PKC may stimulate IGFBP-1 production.

Effects of Fasting, Insulin, and IGF-I. Adult rats fasted for 24 or 48 hr responded with a 10-fold increase in hepatic IGFBP-1 mRNA levels (219). These increased levels returned to baseline after a 24-hr refeeding period. Hepatic IGFBP-1 mRNA and serum IGFBP-1 levels were also increased in growth-retarded fetal rats and sheep deprived of adequate nutrition *in utero* due to maternal fasting (219, 220) or uterine artery ligation (127, 128).

Adult rats made insulin deficient (diabetic) by administration of streptozotocin responded with increased levels of both serum IGFBP-1 and hepatic IGFBP-1 mRNA (193, 221). Recent studies demonstrate that hepatic IGFBP-1 mRNA levels rise in these diabetic rats due to a marked increase in gene transcription, and that treatment of these rats with insulin results in a prompt fall in IGFBP-1 serum protein and hepatic mRNA levels in association with a marked decrease in the rate of hepatic IGFBP-1 gene transcription (222). A similar effect has been noted in diabetic rat kidney (223).

Insulin also inhibits IGFBP-1 *in vitro* (110, 224–226). As little as 0.3 nmol/liter insulin decreases IGFBP-1 accumulation in medium conditioned by human fetal liver explants; maximal inhibition of 50% was noted with 30 nmol/liter insulin (170). Physiologic concentrations of insulin also inhibited IGFBP-1 protein accumulation and IGFBP-1 mRNA levels in rat H35 hepatoma cells and the H4IIE subline, rat primary hepatocytes, and in HepG2 human hepatoma cells (70, 110, 202, 206–208, 225). In HepG2 cells, insulin impaired new IGFBP-1 protein synthesis as measured by metabolic labeling (110), and the inhibitory effect of insulin has been shown to be mediated through the insulin and not the Type I IGF receptor (226).

In H35, HIIE, and HepG2 cells, the inhibitory effect of insulin is mediated at the level of transcription (70, 110, 206, 208) with no evidence of an effect on mRNA stability (110, 207). In HepG2 cells, this effect is conferred by an insulin-responsive element which is located in the human IGFBP-1 promoter between 120 and 96 bp 5′ to the mRNA cap site and which is 100% conserved in the rat promoter. Recent studies demonstrate that this insulin-responsive element confers insulin responsiveness to the otherwise insulin nonresponsive thymidine kinase promoter (227). Insulin inhibition of IGFBP-1 is dominant to (i) dexamethasone and cAMP effects on IGFBP-1 mRNA and protein levels (110, 202, 206, 207), (ii) dexamethasone effects on IGFBP-1 transcription rate (170), and (iii) dexamethasone and cAMP effects on IGFBP-1 promoter activity (110, 208).

IGF-I has no effect on IGFBP-1 expression in either human fetal liver explants (170) or cultured primary rat hepatocytes (202). However, IGF-I and IGF-II are at least equipotent with insulin in inhibiting IGFBP-1 protein, mRNA, and promoter activity levels in HepG2 cells (224, 228, 229). This effect is apparently mediated by the Type I IGF receptor since IGF-II has higher affinity for the Type I IGF receptor than for the insulin receptor (230, 231). It is not known whether IGF-I or IGF-II have similar effects on normal liver *in vivo*, although IGF-I had no effect on IGFBP-1 production in human fetal liver explants (170). Inhibitory effects of IGF-I and IGF-II on IGFBP-1 have been observed in human endometrial and granulosa-luteal cells (232–235).

Uterus

Ovulatory Cycle and Pregnancy. Studies in rats and baboons show that, similar to humans, uterine IGFBP-1 protein and mRNA are detectable only during the secretory phase of the ovulatory cycle, with maximal uterine expression occurring in the decidualized endometrium of pregnancy (71, 101, 236). Expression of IGFBP-3, but not IGFBP-1, is found in the placenta. In baboon, estrogen and progesterone act synergistically to increase IGFBP-1 levels, and the data suggest that the natural cyclic fluctuations of progesterone may be important for maximal IGFBP-1 expression (101). Unlike humans, where uterine IGFBP-1 expression is confined to decidual cells during both the menstrual cycle and pregnancy, baboons express IGFBP-1 primarily in the epithelial cells of the deep basal glands during the luteal phase. During baboon pregnancy, the site of epithelial IGFBP-1 expression moves from the deeper glands to the placental/endometrial junction and then, in late pregnancy, into the decidual stroma (236). The first stromal cells to express IGFBP-1 are located near this junction, often surrounding local spiral arteries. It is postulated that in the baboon, where oocyte implantation is superficial, the pattern of IGFBP-1 expression in the deeper glands during early pregnancy may serve to augment IGF action at the site of implantation and facilitate trophoblast contact with the maternal circulation. Later overexpression of IGFBP-1 in the more superficial layers may prevent continued trophoblast invasion. This contrasts with humans, where implantation is deep and the trophoblast invades aggressively; in this instance, IGFBP-1 expression by the decidua may serve to block IGF stimulation of trophoblast growth.

Extrauterine tissues that display characteristics of uterine decidual endometrium have also been reported to express IGFBP-1 protein (237).

Hormonal Regulation of Uterine Endometrium *In Vitro*. The differentiation of endometrial stroma into decidua seems to require early exposure to estrogen with subsequent exposure to progesterone. Additional factors, probably absent during the secretory phase of the menstrual cycle but present during pregnancy, are required to stimulate cell hypertrophy and enhance decidualization. One of the characteristics of decidual cells is their ability to express IGFBP-1; indeed, IGFBP-1 is the major protein secreted by fully decidualized stromal cells (212). IGFBP-1 is not expressed by nondecidualized endometrium *in vitro*. However, in the presence of progestins, these cells demonstrate increased transcription of the IGFBP-1 gene, accumulation of IGFBP-1 mRNA, and increased synthesis and secretion of IGFBP-1 protein (89, 212, 235). The effect of progestins is not immediate; even after 14 days, IGFBP-1 is not the major secreted protein. However, exposure of progestin-primed cells to relaxin increases IGFBP-1 mRNA level and protein synthesis such that IGFBP-1 becomes the major secreted protein. The effects of relaxin may be mediated through cAMP, and cAMP has been demonstrated to stimulate IGFBP-1 production by decidual cells *in vitro* (232).

Interestingly, both progestin withdrawal and addition of the progestin antagonist RU486 result in transient, marked increases in IGFBP-1 expression similar to that seen with relaxin, indicating that regulation of decidual IGFBP-1 production is complex and not the result of simple transcriptional activation by progesterone (89, 212, 234). In addition, both insulin and IGF-I decrease IGFBP-1 mRNA and protein accumulation in decidual cells (232, 234, 235). Similar to observations in HepG2 human hepatoma cells, the inhibitory effects of insulin and IGF-I appear to be dominant over the stimulatory effects of cAMP and relaxin in decidual cells.

Ovary

Rat ovary contains IGFBP-2, -3, and -4 mRNA, but IGFBP-1 transcripts have not been detected (238). Small amounts of IGFBP-1 protein have been detected in the human ovary, localized to the luteinized granulosa cells of the corpus luteum and hyperstimulated follicles, but not in preovulatory, nonluteinized follicles (239–242). IGFBP-1 mRNA expression and protein production has been found in human ovarian granulosa cells (91, 104, 243).

Jalkanen *et al.* (215) studied the regulation of IGFBP-1 levels found in medium conditioned by cultured human granulosa-luteal cells. Low basal IGFBP-1 levels were significantly stimulated by both phorbol ester and prostaglandin E_2, implying independent regulatory mechanisms involving PKC and adenylate cyclase, respectively. The physiologic significance of this regulation is poorly understood; however, these studies suggest that IGFBP-1 may play a role in regulating human ovarian function (239, 244).

Kidney

IGFBP-1 mRNA is present in rhesus monkey kidney and expression levels parallel the developmental pattern observed in liver; i.e., mRNA levels are first detectable during late gestation, peak during the neonatal period, and are low or undetectable in the adult (F. Liu, D. Styne, R. L. Hintz, and D. R. Powell, unpublished observations). IGFBP-1 mRNA has not been identified in human kidney (245).

Recent *in situ* hybridization studies localize IGF-I and IGFBP-1 mRNA to epithelial cells lining the medullary thick ascending limb of Henle's loop (TAL) in the rat (100). IGF-I receptor mRNA is found in this same region, as well in the distal nephron and glomerulus. After hypophysectomy, IGF-I mRNA levels fall

and IGFBP-1 mRNA levels rise, whereas reverse effects occur with GH replacement. This striking reciprocal regulation, noted even at the level of individual nephrons, suggests that IGF-I expression inhibits IGFBP-1 expression in TAL, similar to the inhibitory effects of IGF-I in uterine endometrium and HepG2 cells. Based on distribution and regulatory patterns, Chin *et al.* (100) postulate that IGF-I and IGFBP-1 play a role in distal renal tubular function. As mentioned in a preceding section, insulin regulation of IGFBP-1 in diabetic rat kidney has also been reported (223, 246).

IGFBP-1 Function

The availability of purified IGFBP-1 has enabled numerous studies of its *in vitro* bioactivity. To date, virtually all of the known actions of IGFBP-1 are related to its ability to specifically bind and modulate the actions of the IGF. However, Liu *et al.* (247) have reported that IGFBP-1 and, even more potently, IGFBP-3 inhibit serum-stimulated DNA synthesis by chick embryo fibroblasts, and it appears this effect may, in part, be independent of IGF binding.

As measured by charcoal or pregnancy-associated endometrial α_1-globin-separated ligand binding assay, the affinity of IGFBP-1 for ^{125}I[IGF-I] is estimated to have a K_d of 10^{-8}–10^{-10} mole/liter (18, 248–250), greater than or equal to radiolabeled IGF-I affinity for the Type I IGF receptor, and considerably higher than IGF-I affinity for the insulin or Type II IGF (IGF-II) receptor. IGFBP-1 has nearly identical affinity for radioiodinated IGF-II (18). It is not known how closely radiolabeled IGF mimic native IGF in terms of receptor or IGFBP affinity. For insulin, iodination is known to significantly affect receptor affinity (251). However, assuming that the relative affinities for receptors and IGFBP are closely approximated using radioiodinated IGF, it appears that free IGF may bind at least equally to IGFBP-1 as to cell membrane receptors. Furthermore, as discussed in a previous section, posttranslational serine phosphorylation of IGFBP-1 may increase its affinity for IGF by as much as 7-fold (84, 85, 252).

Each IGFBP-1 molecule is postulated to have a single IGF binding site (18, 248–250). The binding domain in the IGFBP-1 molecule is not fully characterized but, as discussed in a preceding section, appears to require cooperation between determinants in the amino (Region 1)- and carboxy (Region 3)-terminal ends of the molecule. The midregion probably has no effect on IGF binding.

The corresponding binding site on the IGF molecules appears to have both B and A chain determinants (248, 249, 253, 254). The native structures of IGF-I and IGF-II contain three disulfide bridges (254), two of which are crucial for IGFBP and receptor binding (249). Des-(1–3)-IGF-I, lacking the three amino-terminal amino acids of IGF-I, has essentially no binding to IGFBP-1 (248). Mutations or deletions of amino acids 3 and 4 (IGF-I: pro-glu, IGF-II: ser-glu) of the B chain and 49, 50, and 51 (arg-ser-cys) of the A chain of IGF-I likewise have adverse effects on IGFBP-1 binding (247, 248). By three-dimensional interactive molecular graphics (254) and by solution nuclear magnetic resonance (255), these regions of the B and A chains are predicted to be closely approximated and may comprise a major portion of the IGFBP binding domain. As determined by studies using IGF analogs, this putative IGFBP-1 binding site is similar or identical to that for IGFBP-2, and apparently not identical with the sites involved in IGFBP-3, -4, or -5 or the insulin and IGF receptors (248, 249). Since IGFBP-1 appears to inhibit IGF binding to cell surface receptors, it is possible that a conformational change following IGF/IGFBP-1 association masks or alters the receptor binding site on the IGF molecule (249).

In vitro, the IGF/IGFBP-1 complex appears to be stable when studied under serum-free conditions. IGFBP-1 has been found to inhibit IGF-I binding to numerous mammalian cells, including human granulosa-luteal cells (256), human choriocarcinoma cells (257), human secretory phase endometrial cell membranes (258), human fetal skin fibroblasts (259), FRTL5 rat thyroid follicular cells (260), bovine skin fibroblasts (261), chick embryo fibroblasts (262), and chick pelvic cartilage (263). Inhibition of IGF binding has been correlated with inhibition of IGF-mediated mitogenesis in chick fibroblasts (262), chick pelvic cartilage (263), FRTL5 cells (260), and human granulosa-luteal cells (256), as well as inhibition of IGF-mediated amino acid uptake in human choriocarcinoma cells (257). Addition of IGFBP-1 without IGF-I also inhibits growth of chick pelvic cartilage explants, presumably due to inhibition of endogenously produced IGF (263).

Two groups have reported that IGFBP-1 may enhance IGF-I-mediated actions *in vitro*. Elgin *et al.* (264) reported that addition of a purified IGFBP-1 fraction from amniotic fluid markedly enhanced IGF-I-stimulated mitogenesis in porcine aortic smooth muscle cells, chick embryo fibroblasts, mouse embryo fibroblasts, and human fibroblasts. The authors postulated a possible effect of IGFBP-1 to enhance cell membrane association of IGF-I. IGFBP-1 multimer formation (265) and/or phosphorylation (84, 85, 252) may also play a role in mediating this effect. It is apparent that the enhancement may depend on the presence of platelet-poor plasma (264). One possible explanation is that limited proteolysis of the IGFBP-1/IGF complex by plasma components may be leading to gradual release of free IGF into the cell culture medium, as has been suggested for IGFBP-3 (266). Koistinen *et al.* (259) have reported an apparently paradoxical inhibition of IGF-I binding and enhancement of IGF-I action by

IGFBP-1 in human fetal skin fibroblasts, an observation which remains unexplained.

There are only limited data regarding IGFBP-1 action *in vivo*. As summarized in a previous section, elevated IGFBP-1 levels in intrauterine growth retardation (124, 127), chronic renal failure (58), and GH deficiency (153), and decreased levels in obesity (164) are consistent with an IGF-inhibitory role for IGFBP-1. In the only direct *in vivo* study published to date, infusions of human IGFBP-1 into rats inhibited the hypoglycemic effect of equimolar infusions of human IGF-I (188). Interestingly, infusion of IGFBP-1 alone caused a small but significant increase in blood glucose levels, implying that a basal free fraction of IGF-I exerts a tonic hypoglycemic effect. Insulin levels were not measured in this study.

After IGF binding to IGFBP-1 *in vivo*, the complex is presumably cleared rapidly by the same mechanism by which IGFBP-1 itself is cleared. This is supported by data showing no influence of IGF-I on clearance of human IGFBP-1 infused into rats (188). Furthermore, although radioiodinated IGF can be cross-linked to IGFBP-1 in human plasma isolates, a similar native complex has yet to be demonstrated. Most of the serum IGFBP-1 present *in vivo* appears to be unsaturated, and a direct correlation of IGFBP-1 levels and unsaturated IGF binding has been reported (58). Infusion of IGF-I into rats or humans (267, 268) shows rapid association with a lower molecular mass IGF-binding protein followed by movement of the IGF into a higher molecular mass complex and increased levels of this complex. Together, these data suggest that the IGF/IGFBP-1 complex exists only transiently in plasma.

A Model for IGFBP-1 Function

As summarized above, the bulk of *in vivo* and *in vitro* data indicates that IGFBP-1 may play a role in glucose counterregulation. A more complete model for the physiologic function of IGFBP-1 should, at a minimum, include the interactions of IGF-I and IGF-II with all six known IGFBP as well as the insulin and types I and II IGF receptors. Since this information is largely incomplete, a more reasonable approach is to develop a working model based on the available data. Such a model can provide a framework for further investigation, but does not exclude the possibility of other, as yet uncharacterized, actions of IGFBP-1.

The assumptions included in this model are:

1. The bioactivity of IGFBP-1 is dependent upon its ability to bind IGF.
2. The primary bioaction of IGFBP-1 is to inhibit binding of IGF to cell membranes.
3. IGFBP-1 is the only one of the six IGFBP that shows dynamic regulation in relation to substrate availability.
4. This dynamic regulation is primarily due to rapid and potent insulin-suppression of IGFBP-1 transcription and translation in the presence of a rapid, relatively constant, IGFBP-1 clearance.
5. The distribution space of IGFBP-1 includes the intravascular fluid and lymph.
6. The primary production site for plasma IGFBP-1 is the liver.

Based on these assumptions, we propose that serum IGFBP-1 plays a role in regulating the metabolic actions of the free IGF fraction. As pointed out by Baxter and Martin (1), although most of the plasma IGF is associated with a static complex containing IGFBP-3 and ALS, the estimated 2–4% of total IGF that is unbound (267–269) could theoretically have a significant hypoglycemic effect. In the fed state, during which IGFBP-1 levels are low, IGF effects on substrate utilization may supplement the actions of insulin. Conversely, and possibly more importantly, during fasting, elevated IGFBP-1 levels may inhibit IGF effects on substrate utilization. This postulated action is consistent with the estimated concentrations of IGFBP-1 in postnatal fasting serum (2–10 pmol/liter) and the 1–2 pmol/liter of estimated total free IGF. Moreover, recent data show an inverse correlation of free IGF-I and IGFBP-1 in diabetic rats (270).

Furthermore, the data summarized in this review, including human and animal *in vivo* and *in vitro* studies, indicate that counterregulatory hormones, such as glucocorticoids and glucagon, stimulate IGFBP-1 expression, whereas insulin plays a central and dominant regulatory role by inhibiting this expression. Regulation of IGFBP-1 appears to be at the level of gene transcription, and parallels that of gluconeogenic enzymes, such as phosphoenolpyruvate carboxykinase (271, 272), suggesting a role for IGFBP-1 in glucose counterregulation. This possibility is supported by the recent observation that acute infusion of IGFBP-1 into rats results in a transient increase in serum glucose (188). By binding free serum IGF, IGFBP-1 may thereby inhibit IGF-mediated glucose uptake and/or utilization.

The model described above includes only the actions of IGFBP-1 in serum. Possible paracrine or autocrine roles for IGFBP-1 at or near its sites of production (e.g., liver, uterus, kidney) also deserve consideration. However, current data are too limited to construct a model for the local regulation and actions of IGFBP-1.

Summary

IGFBP-1 is one of six structurally similar IGFBP identified in humans and other mammals. IGFBP-1 has a predicted molecular mass of 25.3 kDa and has cysteine-rich N- and C-terminal regions that are in-

volved in IGF binding. Of the six IGFBP, IGFBP-1 has the unique characteristic of rapid *in vivo* regulation in plasma in relation to meal intake. This phenomenon appears to be primarily due to transcriptional control by insulin coupled with a rapid clearance rate. In humans, IGFBP-1 is primarily produced by the liver.

In vivo and *in vitro* studies indicate that insulin-suppression of IGFBP-1 transcription is dominant over all stimulatory factors except for phorbol esters. The physiologic significance of this regulation is incompletely defined. However, cortisol and glucagon stimulate IGFBP-1 production, implying that IGFBP-1 may play a role in glucose counterregulation. Limited *in vivo* data support this hypothesis.

In vitro, IGFBP-1 appears to function primarily to inhibit IGF-mediated mitogenesis and metabolism. *In vivo*, elevated plasma IGFBP-1 levels in intrauterine growth retardation, chronic renal failure, fasting and growth hormone deficiency, and low levels in obesity are consistent with a postulated role for IGFBP-1 in modulating IGF-mediated substrate utilization. Since the regulation of IGFBP-1 is very responsive to insulin, measurement of IGFBP-1 levels may be useful as a measure of hepatic insulin sensitivity, although the relationship of IGFBP-1 response to other markers of insulin sensitivity require additional investigation. Plasma IGFBP-1 levels may eventually be useful in monitoring of fetal and childhood growth, treatment of obesity, insulin replacement in diabetes mellitus, and liver abnormalities.

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