MINIREVIEW

Insulin-Like Growth Factor Binding Protein–1: Recent Findings and New Directions (44182)

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Abstract. **In 1988, insulin-like growth factor–binding protein–1 (IGFBP-1) became the first characterized member of a group of structurally related soluble proteins which specifically bind and modulate the actions of the IGFs. Since then, a wealth of information has accumulated regarding the physiology of this dynamic serum protein. In this review, we update our 1993 summary (Lee PDK et al. Proc Soc Exp Biol Med 204:4–29) of the status of IGFBP-1 research.**

The IGFBP-1 protein sequence contains 12 N-terminal and 6 C-terminal cysteine residues which are conserved in other mammalian IGFBP-1 sequences and amongst other IGFBPs; both of the cysteine-rich regions are required for optimal IGF binding. The nonconserved IGFBP-1 midregion may act as both a hinge which defines ligand binding characteristics and as a specific target for protease activity. Integrin-binding and phosphorylation sites within the IGFBP-1 sequence have functional significance *in vitro,* but their physiologic relevance *in vivo* have not been defined. The human IGFBP-1 and IGFBP-3 genes are contiguous and located in close proximity to the homeobox A (HOXA) gene cluster on chromosome 7. The other IGFBP genes, located on chromosomes 2, 12, and 17, are also associated with HOX clusters, suggesting evolutionary linkage of the IGFBP and HOX gene families. Similarities between the hIGFBP-1 and phosphoenolpyruvate kinase (PEPCK) promoters, including regions conferring insulin, glucocorticoid, and cyclic adenosine-monophosphate responses, are consistent with our previous hypothesis that IGFBP-1 is involved in regulation of glucose metabolism. The tissue-specific patterns of IGFBP-1 gene expression in liver, kidney, decidua, and ovary may be due to stimulation of IGFBP-1 transcription by hepatic nuclear factor 1 (HNF1) proteins.

Clinical and basic studies of IGFBP-1 physiology have been aided by several recently developed assay methods. Numerous investigations have confirmed that insulin, *via* inhibition of IGFBP-1 transcription, is the primary determinant of IGFBP-1 expression both *in vitro* and *in vivo.* IGF-I and IGF-II also have specific inhibitory effects on IGFBP-1 expression. Glucocorticoids and cAMP stimulate IGFBP-1 transcription, but these effects are observed only in conditions of low or absent insulin effect. Other stimulants of IGFBP-1 expression include thyroid hormones and epidermal growth factor. Phorbol ester stimulation of IGFBP-1 expression can supersede the effects of insulin *in vitro;* however, the mechanism and *in vivo* correlates of this effect have not been determined. Cytokines and, perhaps, growth hormones may affect IGFBP-1 expression, perhaps by altering the regulatory actions of insulin; this effect may have important clinical relevance. IGFBP-1 expression is upregulated in liver and (nonhuman) kidney during postinjury regeneration.

The IGF-inhibitory actions of IGFBP-1 has been confirmed by numerous *in vitro* studies and several *in vivo* animal investigations, including administration of recombinant IGFBP-1 and IGFBP-1 transgenic models. IGFBP-1 has been shown to inhibit somatic linear growth, weight gain, tissue growth, and glucose metabolism. Moreover, IGFBP-1 appears to be a primary determinant of free IGF-I levels in serum. Excess levels of IGFBP-1 may contribute to growth failure in intrauterine growth restriction and in pediatric chronic renal failure, while low IGFBP-1 levels are associated with

obesity and with cardiovascular risk factors in insulin resistance syndromes. Serum IGFBP-1 measurements may be useful biochemical marker in these pathologic conditions.

IGFBP-1 is expressed in decidualized stromal cells of the uterine endometrium and in ovarian granulosa cells. IGFBP-1, together with IGFs, insulin, ovarian steroids, cytokines, and other factors, is involved in a complex system which regulates menstrual cycles, ovulation, decidualization, blastocyst implantation, and fetal growth. Models for the role of IGFBP-1 in female reproductive physiology are presented, and evidence for pathophysiologic roles in pre-eclampsia, polycystic ovarian syndrome, and uterine malignancy are reviewed.

Very recent data indicates that IGFBP-1 undergoes regulated expression in human osteoblasts. Limited information also suggests that IGFBP-1 may be present in peripheral neurons, and that serum IGFBP-1 may increase during exercise and in critical illness.

In summary, two major roles for IGFBP-1 in normal physiology can be constructed from current data: (i) As an "endocrine" factor, IGFBP-1 regulates the bioavailability of serum IGF-I, thereby modulating IGF-mediated tissue metabolism. The dominant regulation of IGFBP-1 expression by meal-related changes in hepatic insulin concentrations provides a dynamic link to substrate availability. (ii) As an autocrine/paracrine factor, IGFBP-1 appears to play a crucial role in the female reproductive system and, in particular, the sequence of events leading from ovulation to implantation to successful fetal outcome. Future investigations will further delineate the manner in which IGFBP-1 participates in these and other physiologic processes, and the mechanisms by which IGFBP-1 may be involved in clinical pathophysiology.

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With the report of its complementary deoxyribonucleic acid (cDNA) sequence in 1988 (1), insulin-like growth factor–binding protein–1 (IGFBP-1) was the first characterized member of a family of soluble proteins which specifically bind and modulate the effects of insulin-like growth factor–I and II (IGF-I and II). Five additional IGF-binding proteins have been subsequently identified and, by convention (2), have been numbered IGFBP-2 through 6 according to the sequence of their published report. A seventh protein with sequence homology to the IGFBPs has recently been described (3), and additional members of this protein family may be identified in the future. IGFBP-1 through 6 are related to one another by structural and functional homologies, but have clearly distinct physiologic characteristics.

In 1993, we had the opportunity to review the status of IGFBP-1 research (4).[2] At that time, it was already evident that IGFBP-1 is unique amongst the IGFBPs in having dramatic minute-to-minute kinetics in the peripheral circulation. This characteristic is primarily due to a rapid endogenous clearance rate coupled with potent insulin inhibition of IGFBP-1 transcription (reviewed in Ref. 4). The inverse relationship of IGFBP-1 to insulin and various other lines of evidence suggested that IGFBP-1 might play roles in glucoregulation and in the regulation of IGF-mediated substrate utilization. In the nearly 4 years since our review, considerable supportive evidence for these hypotheses has accumulated and other physiologic roles for IGFBP-1 have been further defined. In this paper, we review recent progress in IGFBP-1 research. For the sake of brevity, we will frequently refer back to our previous review (4) for literature cited therein.

Molecular Characterization

IGFBP-1 Protein Organization. Detailed characterization of the IGFBP-1 protein structure was reported previously (4) and will be briefly reviewed here with emphasis on recent findings. The complete amino acid sequence of human IGFBP-1 (hIGFBP-1) was predicted from the cDNA sequence and confirmed by direct sequencing of purified IGFBP-1 protein (4). In addition, as shown in Figure 1, IGFBP-1 protein sequences from rat, cow, and mouse have been predicted from their cDNAs (4, 5). Not depicted in Figure 1 are the extremely hydrophobic N-terminal signal peptides required for cellular secretion of IGFBP-1.

The primary amino acid sequence of IGFBP-1 is remarkable for conservation of 12 N-terminal and 6 C-terminal cysteine residues. These 18 cysteines and their spatial orientation are conserved in all IGFBP-1 through IGFBP-5 forms characterized to date. On the other hand, IGFBP-6 sequences lack some cysteines in the N-terminal cluster (4). All 18 cysteines of IGFBP-1 participate in intrachain disulfide linkages, although the specific cysteine

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[2] In Table 1 of this article, sample timing for fetal serum was incorrectly labeled. The unit of measurement for the four entries should be *days*, not *weeks*.

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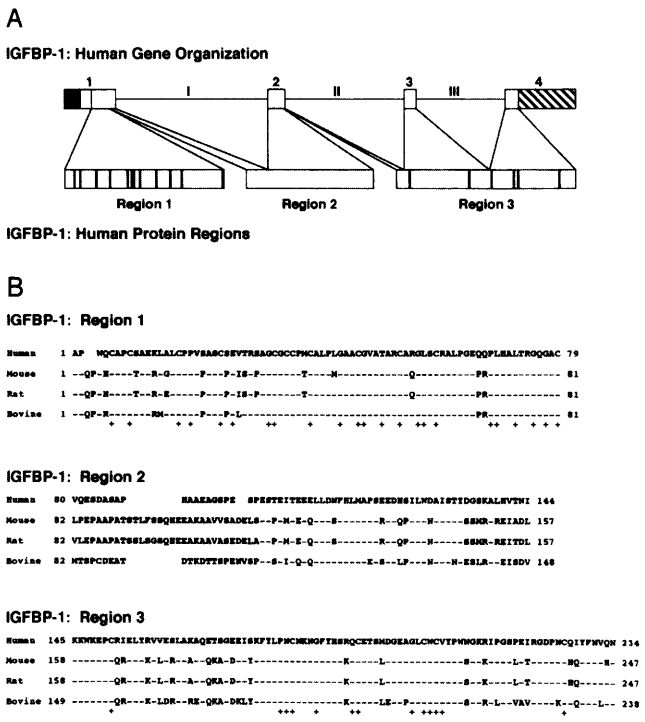

Figure 1. Schematic representation of the IGFBP-1 chromosomal gene, cDNA, and protein.

pairings have not been fully characterized. These disulfide linkages apparently provide a structural framework, while surrounding residues provide functional specificity for IGF binding and other actions (4).

The IGFBP-1 protein can be divided into three regions based on the cysteine clusters (4). Region 1 contains the first 79 residues of hIGFBP-1 including the 12 N-terminal cysteines; 61 of these 79 residues (77%) are conserved in rat, cow, and mouse (5). In contrast, only 24 of the 65 (37%) residues in region 2 (i.e. hIGFBP-1 [80–144]), are conserved between these species, and this region is not homologous with region 2 from any of the other five IGFBPs. Region 3, spanning residues 145 through 234 of hIGFBP-1, contains the 6 C-terminal cysteines; 59 of these 90 residues (60%) are conserved in rat, cow, and mouse IGFBP-1.

A number of studies suggest that the IGF-binding domain of IGFBP-1 is diffuse, and that regions 1 and 3 are both required for optimal binding of IGF peptides (4). It is likely that region 2 acts as a hinge which allows the protein to fold back upon itself such that regions 1 and 3 can interact with a single IGF ligand. This model may explain the similarities and differences between the IGFBPs. For example, regions 1 and 3, which are homologous between IGFBPs, provide fundamental ligand specificity, while region 2, which is not conserved, acts as a hinge which modifies ligand binding affinity and specificity. Recent studies indicate that differences in the specificity of the IGFBP for IGFs are probably not primarily dependent upon structural determinants in the IGF molecules themselves (6, 7), further emphasizing the importance of region 2 differences.

Region 2 may also serve as a target for IGFBP proteases with differences in this region conferring IGFBP-specificity for individual protease action. Region 2 of IGFBP-1 is rich in proline (P), glutamine (E), serine (S), and threonine (T) residues, and contains a defined PEST domain which could increase the susceptibility of IGFBP-1 to proteolysis. In contrast, a PEST domain is not found in the other IGFBPs; however, a wide range of proteases may specifically target other region 2 sites in IGFBP-1 and the other IGFBPs (8–12). Hypothetically, proteolysis in the hinge region (region 2) would separate the ligand binding domains (regions 1 and 3), effectively lowering the affinity of IGFBPs for IGF ligand. Thus, proteases may modulate the release of IGF peptides to target tissues, with inter-IGFBP differences in the region 2 sequence defining the relative responsiveness to different proteases.

Region 3 of hIGFBP-1 contains an Arg-Gly-Asp (RGD) sequence which is conserved in rat, cow, and mouse IGFBP-1 (Fig. 1) and in rat and human IGFBP-2 (4, 5). RGD sequences are present in a group of extracellular matrix proteins and mediate binding of these proteins to specific cell surface receptors known as integrins (13). *In vitro* studies demonstrate that IGFBP-1, *via* its RGD sequence, binds to $\alpha_5\beta_1$ integrin, also known as the fibronectin receptor, and allows IGFBP-1 stimulation of cell migration in cultures of Chinese hamster ovary (CHO) cells, porcine smooth muscle cells (pSMC) (14), and, perhaps, human trophoblast cells (15). This action is probably mediated by IGFBP-1 blockade of fibronectin binding; fibronectin is known to inhibit cell migration (16, 17). Conversely, IGFBP-1 can inhibit pSMC migration by preventing the more potent cell-migration stimulants IGF-I and IGF-II from interacting with their cell membrane receptors (15, 18). Finally, in MDA-MB-231 and MG-63 cells, both of which express $\alpha_5\beta_1$ integrin, association of either endogenous or exogenously-added IGFBP-1 with $\alpha_5\beta_1$ integrin could not be demonstrated and IGFBP-1 did not inhibit cell attachment to fibronectin-coated surfaces (19). Thus, if IGFBP-1 plays an *in vivo* role in cell attachment and migration, it is likely to be a complex process.

Although the IGFBP-1 sequence contains a possible site for heparin binding (20), recombinant IGFBP-1 had no significant affinity for heparin-Sepharose and there was no effect of heparin on IGFBP-1 binding to radio-iodinated IGF-I (21, 22).

IGFBP-1 is secreted as a phosphoprotein with serine residues at positions 101 (70% of total phosphorylation), 169 (25%), and 119 (5%) serving as the phosphorylation sites (23–26). Ser-101 and Ser-119 are conserved in rat, cow, and mouse IGFBP-1, but Ser-169 is not (Fig. 1). *In vitro* studies suggest that phosphorylation of IGFBP-1 may be associated with increased affinity for IGF peptides since mutation of Ser-101 to Ala-101 prevents phosphorylation of this residue and results in a 3-fold decrease in affinity for IGF-I (23). As reviewed in a later section, there is evidence that IGFBP-1 isoform patterns change in certain clinical conditions (27–29).

IGFBP-1 Chromosomal Gene Organization and Evolution. The organization of the chromosomal gene for hIGFBP-1 is shown schematically in Figure 1 (4). The chromosomal genes for rat and mouse IGFBP-1 have also been described (5, 30). Each of these three IGFBP-1 genes is roughly 5 kb in length and contains four exons separated by three introns. This is similar to the organization of the other five human IGFBP genes with the exception of IGFBP-3, which has a fifth exon present in the 3′-untranslated region (31–33). The exon 1/exon 2 border is identical in rat and mouse genes but differs from the hIGFBP-1 gene; this border is also variable among the human genes for IGFBP-2 through 6. In contrast, the exon 2/exon 3 and exon 3/exon 4 borders are identical among the three known IGFBP-1 genes and among the human genes for the other IGFBPs (5, 30, 32, 33). Introns 1, 2, and 3 vary in size and sequence among the rat, mouse, and human IGFBP-1 genes. However, a ~300 bp region of intron 1 is conserved among the three genes. This region, corresponding to bp +910 to +1238 of hIGFBP-1, is 80% identical in the rat and mouse genes (5, 30). The high degree of sequence conservation suggests an important function, perhaps in the regulation of IGFBP-1 gene transcription. Indeed, potential DNA-binding sites for a number of transcription factors have been identified in this region (5).

The hIGFBP-1 gene has been localized to chromosome 7p14–p12, where it borders the hIGFBP-3 gene in a tail-to-tail orientation (32). At present, there is no evidence that these genes share regulatory elements in their intergenic regions. This arrangement is similar to that of hIGFBP-2 and hIGFBP-5, which border each other in a tail-to-tail orientation on chromosome 2q33–q34 (34, 35).

Investigation of the evolution of the IGFBP gene family was aided greatly by the observation that the human IGFBP genes are closely linked to the homeobox (HOX) genes. HOX genes, which are present in organisms ranging from yeast to humans, encode transcription factors which are important in early morphogenesis (36). Both of the gene families are localized to four chromosomal regions: 7p15–p12 includes IGFBP-1 and 3 and the HOXA cluster, 2q31–q34 includes IGFBP-2 and 5 and the HOXD cluster, 17q12–q22 includes IGFBP-4 and the HOXB cluster, and 12q13 includes IGFBP-6 and the HOXC cluster (32, 35). Phylogenetic studies indicate that duplication of HOX genes at a single chromosomal locus apparently preceded dispersion of the resulting HOX cluster to the current four chromosomal loci (36). Close associations of hIGFBP and HOX gene clusters suggest that the ancestral HOX and IGFBP genes were linked prior to this initial duplication. In support of this hypothesis, multiple HOX clusters have been identified in agnathans, which diverged from the main line of vertebrate evolution ~550 million years ago, and IGFBP activity has been recently demonstrated in agnathan serum (37). Furthermore, comparisons of the hIGFBP sequences suggest derivation from an ancestral IGFBP gene which duplicated prior to dispersion to the current four chromosomal loci (reviewed in Ref. 35).

Another protein, originally labeled mac25 (37a), has been designated as IGFBP-7 since it contains 11 of the 12 N-terminal cysteines characteristic of the other IGFBPs and has been shown to bind IGFs on ligand blot (37b). However, several characteristics suggest that mac25 may be only distantly related to the other IGFBPs. In particular, mac25 (i) lacks the C-terminal cysteine cluster which is present in IGFBP-1 through 6, (ii) has a much lower amino acid identity with IGFBP-1 through 6 than these six IGFBPs have with one another, (iii) binds IGFs with much lower affinity than IGFBP-1 through 6, and (iv) is located on chromosome 4q12, a site that does not contain a HOX gene cluster. Therefore, the relationships between mac25 and the other six IGFBPs have not been completely elucidated.

Primer extension studies of IGFBP-1 mRNA from human (HepG2) and rat (H4IIE) hepatoma-derived cells and from human uterine decidual cells reveals the transcription start site at 165 and 174 bp 5′ to the translation start site for human and rat IGFBP-1, respectively (31, 38, 39). Thus, the combined sizes of exons 1–4 in the human and rat genes predict an IGFBP-1 mRNA transcript of ~1.5 kb, consistent with published Northern blots of not only human and rat IGFBP-1, but also RNA from cow, mouse, and monkey. The finding of a single ~1.5-kb transcript in tissues from each of these species suggests that differential splicing of the IGFBP-1 gene is, at best, a rare event (4).

Moreover, the rapid *in vivo* changes in serum IGFBP-1 in response to insulin, probably due to transcriptional regulation coupled with a rapid IGFBP-1 protein clearance rate, suggests that the IGFBP-1 mRNA has a relatively short half-life ($t_{1/2}$). Indeed, when transcription is blocked by actinomycin D in rat H4IIE cells, the $t_{1/2}$ for IGFBP-1 mRNA is estimated to be ~2 hr (40). A labile protein may be involved in this rapid turnover since the $t_{1/2}$ of H4IIE IGFBP-1 mRNA increases to 20 hr in the presence of cycloheximide, which inhibits protein synthesis (40). A similar accumulation of IGFBP-1 mRNA has been noted in human HepG2 hepatoma cells treated with cycloheximide (41). In IGFBP-1 transgenic mice, temporal expression of the IGFBP-1 transgene was unexpectedly found to be regulated in a similar fashion to the endogenous IGFBP-1 genes; this is apparently mediated by regulated expression of a protein which interacts with AU-rich elements in the 3′-untranslated region (42–44).

The IGFBP-1 gene has a TTTATA motif (TATA element) located 30 bp 5′ to the transcription start site (31, 45). This organization is typical for most eukaryocytic promoters in which the TATA element binds the basal transcription machinery, correctly positioning it to allow accurate initiation of transcription in the presence of RNA polymerase II. In fact, the first 3.6 kb 5′ to the hIGFBP-1 transcription site functions as an IGFBP-1 promoter in several human cell systems (e.g., HepG2 hepatoma cells, Hec1B endometrial carcinoma cells, and primary endometrial stromal cells), as

demonstrated by orientation-specific stimulation of the chloramphenicol acetyltransferase reporter gene expression during transient transfection studies (45–51). In addition, the regions 5′ to the transcription start site in the rat (39) and mouse IGFBP-1 (5) genes have been characterized, and 926 bp of the 5′ rat sequence also acts as a promoter in rat H4IIE cells (52, 53). Comparisons of these human, rat, and mouse promoter regions reveal that the major sequence similarity is in the proximal ~300 bp (Fig. 2). As illustrated by the boxes in Figure 2, this region of the hIGFBP-1 promoter has six DNA elements shown to be of functional significance *in vitro*. Box 1 contains the TATA element and box 2 contains a binding site for hepatic nuclear factor 1 (HNF1), a DNA-binding protein which is primarily responsible for basal IGFBP-1 promoter activity in hepatoma cells and is likely to be responsible for the tissue-specific expression pattern of IGFBP-1 (46, 54–56) (*vide infra*). Boxes 3 and 5 contain the glucocorticoid response elements GRE2 and GRE1, respectively, which bind the glucocorticoid receptor (GR) and act synergistically to confer the stimulatory effect of glucocorticoids to the hIGFBP-1 promoter (57). Box 4 contains an element which binds the hepatic nuclear factor 3 (HNF3) family of transcription factors. This element, which is also known as the insulin response element (IRE), since it confers the entire inhibitory effect of insulin on hIGFBP-1 promoter activity, is also necessary for maximal glucocorticoid stimulation of hIGFBP-1 transcription (58–60). Box 6 contains a cAMP response element (CRE), the binding site for CRE-binding protein, which confers cAMP stimulation to the hIGFBP-1 promoter (58). Inspection of Figure 2 reveals that the TATA element, the HNF1 binding region, IRE, and GRE2 sequences are highly conserved among the human, rat, and mouse promoters, suggesting a crucial, evolutionarily conserved role for these gene promoter regions in IGFBP-1 regulation. In support of this hypothesis, the IRE and GRE2 have been found to be essential in conferring

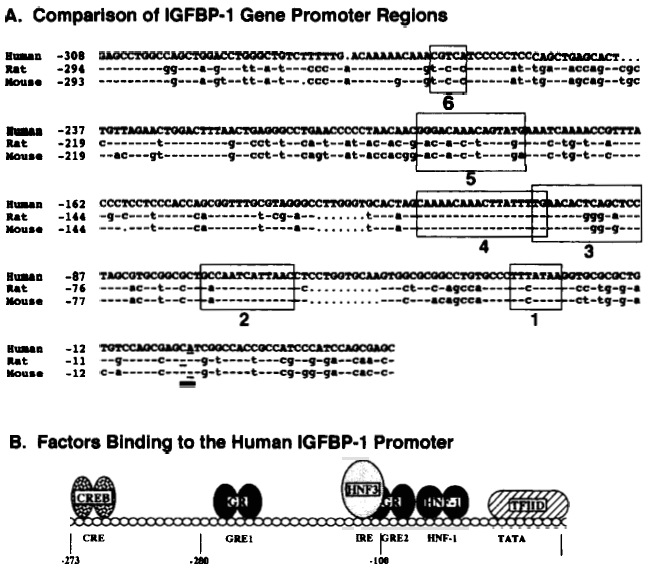

Figure 2. IGFBP-1 gene promoter.

insulin and glucocorticoid effects to the rat IGFBP-1 promoter (52, 53, 61).

A complex transcription regulatory element has been identified ~2.6 kb 5′ to the transcription start site of the hIGFBP-1 gene (49–51). This element contains a central enhancer region which confers a 40-fold stimulation of promoter activity in HepG2, Hec1B, and human endometrial stromal cells. The enhancer is flanked by elements which normally inhibit enhancer action in these cells; this complex appears to play a role in the profound increase in IGFBP-1 transcription during decidualization of endometrial stromal cells (49, 50, 51, 62).

The organization of the IGFBP-1 promoter region is similar to that for phosphoenolpyruvate (PEPCK) (4, 63), a key enzyme involved in the regulation of gluconeogenesis. Both genes are regulated at the level of transcription, with many important *cis*-regulatory elements located within 500 bp of the transcription start site. Comparisons of their organization, hormonal regulation, and tissue-specific expression suggest that although their spatial organizations differ, the PEPCK and hIGFBP-1 promoters use similar *cis*-elements and *trans*-acting factors to achieve similar results (63). This similarity provides further evidence that IGFBP-1 is involved in the regulation of glucose metabolism (*vide infra*).

Tissue-Specific IGFBP-1 Gene Expression. As previously reviewed (4), the IGFBP-1 gene is expressed primarily in liver, decidualized uterine endometrium, ovarian granulosa cells, and kidney. In large part, this expression pattern may be due to stimulation of IGFBP-1 transcription by members of the HNF1 family of proteins. HNF1 forms are responsible for basal IGFBP-1 promoter activity in hepatoma cells (46, 54, 56) and probably also for IGFBP-1 expression in uterine endometrium (49) and renal tubular epithelial cells (64) (*vide infra*). HNF1α was originally described as a master transcription factor involved with producing and maintaining the phenotype of specialized epithelia (64, 65). It appears, therefore, that IGFBP-1 expression may be part of the cellular phenotype which is maintained by HNF1α.

Recent studies have found that mutations in the HNF1α gene are associated with type-3 maturity-onset diabetes of the young (MODY3), suggesting that HNF1α activity may be low both in this disorder and in MODY1 (66, 67). However, transgenic mice lacking HNF1α have abnormal liver function and renal Fanconi syndrome, but they do not have frank glucose intolerance (68). Hence, although HNF1 proteins are necessary for IGFBP-1 promoter activity and IGFBP-1 expression may be decreased in MODY and HNF1α-deficient mice (although this has not yet been studied), a role for the HNF1/IGFBP-1 link in glucose regulation and the pathogenesis of MODY syndromes is uncertain.

IGFBP-1 Assays and Measurements

As previously reviewed, the first quantitative assays for IGFBP-1 were based on the principle of competitive-

displacement radioimmunoassay (RIA) (4, 69): binding of radio-iodinated pure antigen (i.e., IGFBP-1) to antibody is displaced by unlabeled antigen in the sample and compared with a displacement curve constructed with standards containing known amounts of antigen. Homologous (i.e., the purified tracer and/or standards are species specific) RIA methods have been developed for both human (4) and rat IGFBP-1 (70). While competitive RIA methods have great clinical and research utility, they have several recognized limitations, including a primary dependence on the specificity and binding characteristics of the antibody or antiserum, variability introduced by tracer labeling and degradation, and controversies regarding resolution and analysis of the typically sigmoidal displacement curve. In addition, RIA methods are relatively tedious to perform and difficult to automate.

The development of monoclonal antibodies to IGFBP-1 led to development of dual-epitope, direct-detection assays in which the antigen in the unknowns and standards are quantitatively bound to purified antibody, usually coated to a solid surface, and detected using a second antibody preparation which binds to a different site, or epitope, on the antigen. Detection is enabled by radio-iodination of the second antibody preparation (immunoradiometric assay, IRMA) or by use of a nonradioactive enzyme-linked colorimetric-detection system (enzyme-linked immunosorbent assay, ELISA, or enzymometric immunoassay, EIA). Dual-epitope, direct detection methods have relative advantages over competitive RIA, including potentially greater sensitivity and specificity, less complicated assay curve analysis, and more efficient technical performance. However, the use of monoclonal antibodies may result in greater problems with interference by buffer and sample components (assay/sample matrix) as compared with the typical polyclonal antibody RIA. Commercial versions of both IRMA (DSL, Inc., Webster, TX) (71) and ELISA (Medix, Helsinki, Finland) (69) IGFBP-1 kits have facilitated research studies of IGFBP-1, and the dual-epitope method can be easily adapted to automated methods which may employ more sophisticated and sensitive luminescent, fluorescent, electrochemical, or other detection methods.

In our previous review, we discussed the importance of interpreting serum IGFBP-1 concentrations in relation to meal pattern and, in particular, to insulin kinetics, since IGFBP-1 levels show virtually continuous variability which is inversely related to insulin (4). Maternal serum IGFBP-1 levels during labor (72) and certain pathophysiologic states described in later sections may be exceptions to this rule. In cross-sectional population studies, the well-described inverse relationship between serum insulin and IGFBP-1 (4, 73) will be most apparent after a defined period of fasting. For routine sampling, an overnight fast is a typical convenient interval. Recognition of these requirements coupled with improvements in assay methodology have contributed to several recent studies of normal and pathologic ranges.

Ranges for IGFBP-1 as measured in fasting morning samples from 600 normal children by specific, two-site enzyme-linked immunosorbent assay showed an inverse relationship with both age and Tanner pubertal stage (74) with mean concentrations declining more than 5-fold between Tanner stages 1 and 5. A nonlinear inverse relationship was noted between IGF-I, but not IGF-II or IGFBP-3, and IGFBP-1 concentrations. Juul *et al.* have reported similar results in a large population of normal, healthy children and in boys with normal or precocious puberty (75, 76). IGFBP-1 concentrations declined with age and were inversely correlated with the age-related increase in IGF-I, IGF-II, and IGFBP-3 (77). However, IGFBP-1 levels may increase with advanced age, associated with loss of an inverse relationship between fasting IGFBP-1 and insulin (78, 79). Ranges reported in these recent studies are quantitatively similar, but considerably more detailed than those summarized in our previous review (4). Such data provide useful research and clinical guidelines; however, different IGFBP-1 assays can give significantly different results for the same sample. Therefore, interpretation of IGFBP-1 results should always take into account the assay methodology and sample collection conditions.

Recently, controversy has arisen over whether different IGFBP-1 immunoassays may preferentially recognize certain phosphoisoforms. The monoclonal antibodies used in IRMA, ELISA, and related techniques are known to have differing affinities for the various IGFBP-1 phosphoisoforms when used for immunoblot studies, although none of the antibodies exclusively recognize nonphosphorylated or partially phosphorylated forms. The issue is further complicated by the use of recombinant IGFBP-1, which is nonphosphorylated, or amniotic fluid–derived IGFBP-1, which contains a mixture of phosphoisoforms, for assay standardization, while virtually all IGFBP-1 in serum is in a highly-phosphorylated form (24, 28, 29). Indeed, an assay has been recently described which claims to measure "total" IGFBP-1, as opposed to other assays which are conjectured to measure either nonphosphorylated or partially phosphorylated forms (80). The reasoning behind this latter assay is that it gives quantitative estimates of serum IGFBP-1 which are higher than other assays and is unaffected by enzymatic dephosphorylation of the sample; whereas the comparative assay gave an increasing signal in response to sample dephosphorylation.

Although the precise reasons for quantitative differences between assays have not been completely defined, a few points should be considered. First, it would be theoretically difficult, if not impossible, to construct a routine assay which measures *only* nonphosphorylated IGFBP-1. Such an assay would require antibodies which are somehow unable to bind to any of the six possible phosphoisoforms. As an extension of this point, an assay which measures only certain phosphoisoforms would also be very difficult to construct since none of the currently characterized antibodies exclusively recognize one or another phosphoisoform. In addition, dual epitope assays, which use direct-detection

rather than displacement analysis, are less likely to be affected significantly by differences in antibody affinities. Therefore, it seems unlikely that phosphorylation status *per se* could explain observed differences between assays.

A more likely explanation rests in the observation that binding of many, if not most, of the characterized anti–IGFBP-1 antisera and antibodies is inhibited or blocked by IGF-I or IGF-II. IGF may bind to a site at or near the antibody binding site or IGF-binding could induce changes in the secondary or tertiary structure of IGFBP-1, thereby altering or masking the antibody epitope. Phosphorylation may cause a relative shift in the amount of IGF-saturated IGFBP-1 and/or change the relative affinity of IGFBP-1 for IGF versus antibody. Therefore, differences between assays may well be due to differences in IGF saturation, and this may be affected by phosphorylation status. Since IGFBP-1 measurements are now gaining importance as possible clinical measures, further studies of factors which affect quantitative estimation are definitely warranted.

Semiquantitative assays for IGFBP-1 have also seen a few recent advances. A method for investigating IGFBP-1 protein secretion from individual cells using a reverse hemolytic plaque assay has been reported (81). Fowlkes *et al.* (82) recently described a rapid nonradioactive Western ligand blot method using biotin-IGF. Finally, 2-dimensional electrophoresis followed by ligand or immunoblot analysis may allow distinction of individual IGFBP isoforms and quantitative estimation of IGFBP concentrations (83–85).

Regulation of IGFBP-1

Insulin and Metabolic Substrates. Insulin inhibits its hepatic IGFBP-1 expression in rodents and acts through the insulin receptor to inhibit both basal and glucocorticoid-stimulated IGFBP-1 expression in hepatic cells; this inhibition occurs at the level of gene transcription (4, 48, 86–89). Recent studies indicate that insulin, acting through the insulin receptor, also suppresses IGFBP-1 production by primary human ovarian granulosa cells (90) and IGFBP-1 protein and mRNA levels in primary human osteoblast cells (91) *in vitro.* In addition, insulin may inhibit IGFBP-1 expression in kidney (4, 92) and in uterine decidual cells (93).

Insulin inhibition of basal and glucocorticoid-stimulated hIGFBP-1 promoter activity occurs through an insulin response element (IRE) located between −120 and −96 bp 5′ to the transcription start site (57, 89). This 25-bp IRE region, which confers insulin inhibition to the otherwise insulin-nonresponsive thymidine kinase promoter (89) is 100% conserved in the human, rat (39), and mouse (5) IGFBP-1 promoters and is necessary for insulin inhibition of glucocorticoid-stimulated IGFBP-1 promoter activity in the rat (52, 53, 61). The IGFBP-1 IRE contains an inverted palindrome of the sequence T(G/A)TTTTG, which is essential for insulin inhibition (57, 89). The T(A/G)TTT portion of this motif is 100% conserved in the promoters of the PEPCK, tyrosine aminotransferase (TAT), and apolipoprotein CIII (ApoCIII) genes, and in each case this motif is contained in an IRE which confers insulin inhibition to promoter activity (94–96; reviewed in Ref. 97). This suggests that a common transcription factor binds each IRE to confer the inhibitory effect of insulin.

The HNF3 family of transcription factors, which function in hepatic development (98, 99), are candidate proteins for conferring insulin inhibition, since they bind the IGFBP-1, PEPCK, TAT, and ApoCIII IREs (60, 97, 100, 101; Allander SA, Powell DR, unpublished observations). In addition, HNF3 proteins are likely targets of insulin-regulated extracellular signal-regulated kinases (ERKs) and cdc-kinase in liver (102–104). However, despite this suggestive evidence, binding of HNF3 proteins to IRE mutants does not appear to correlate with responsiveness of these mutants to insulin (60, 100). Additional investigations are in progress to define the possible role of HNF3 proteins in IRE function.

In concordance with the molecular data, numerous *in vivo* studies have demonstrated the predominant regulatory effect of insulin on IGFBP-1 (4, 73, 105–113). Concurrent hepatic vein and peripheral vein/artery sampling studies in patients with IDDM have confirmed that insulin potently suppresses hepatic output of IGFBP-1 (114).

Exceptions to the acute inverse relationship between insulin and IGFBP-1 have been reported in a few clinical conditions. During abdominal surgery, serum insulin and IGFBP-1 concentrations were observed to rise concurrently (115). As reviewed in a subsequent section, IGFBP-1 levels also appear to be unrelated to insulin in cases of severe infection, trauma, and burn injury. Cytokine stimulation of hepatic IGFBP-1 production is a proposed mechanism for this phenomenon (116).

A lack of IGFBP-1 response to IGF-I or insulin has been reported in an adolescent with Mendenhall syndrome, a condition of short stature and severe insulin resistance (117). IGFBP-1 levels were relatively high in the basal state despite markedly elevated insulin levels. Infusion of rhIGF-I resulted in decreased insulin concentrations; however, the nadir levels were still several-fold above normal and IGFBP-1 concentrations showed a slight, not statistically significant, decrease. The mechanism for these observations was not defined. Serum IGFBP-1 levels appeared to vary inversely with C-peptide and insulin concentrations, and not with glucose, in an elderly patient with nonislet cell tumor hypoglycemia (118).

In our previous studies, somatostatin and insulin clamp protocols were used to estimate the sensitivity of hepatic IGFBP-1 production to insulin (4). These procedures essentially turn off endogenous pancreatic insulin production, allowing estimation of portal insulin concentrations from peripheral measurements. Using similar procedures, Yki-Jarvinen (119) conducted studies that are consistent with the supposition that IGFBP-1 levels reflect hepatic portal insulin concentrations, but may not be indicative of whole body insulin sensitivity. Further studies will be needed to clarify this issue.

Previous studies have shown that, within physiologic concentration ranges, glucose does not have independent effects on IGFBP-1 *in vitro* or *in vivo* (4, 106, 120). However, glucose availability may modify the *in vitro* suppressive effects of insulin on IGFBP-1 production in primary cultures of rat hepatocytes (121). Although IGFBP-1 responses to insulin infusion have been reported to differ between normal and insulin-dependent diabetic subjects (122), we have previously found no differences in IGFBP-1 kinetics during clamp studies in these populations (73).

Amino acids stimulate IGFBP-1 production *in vitro* (87, 121, 123, 124), perhaps by increasing substrate availability for protein synthesis. However, the *in vivo* relationships of IGFBP-1 and amino acids are likely to be more complex (reviewed in Ref. 125). In rats, both IGF-I and IGFBP-1 are expressed in the renal medullary thick ascending loop; rats fed high-protein diets were found to have increased IGF-I and decreased IGFBP-1 mRNA in this tissue, while low-protein diets caused opposite changes (126). Increased liver and kidney IGFBP-1 mRNA were observed in protein-restricted rats (127–130). Further investigation of the mechanism for increased levels of IGFBP-1 expression in protein-restricted rats showed marked induction of primary IGFBP-1 transcript and a somewhat larger increase in mRNA levels, suggesting a combination of transcriptional and post-transcriptional mechanisms (129). The observed effects of dietary protein restriction may be mediated by changes in insulin secretion; however, insulin was not measured in these studies. In humans, the effects of diet on IGFBP-1 appear to be secondary to changes in insulin concentration. During calorie-restricted diets, IGF-I was clearly related to dietary content and nitrogen balance and not related to insulin, whereas IGFBP-1 was related to insulin rather than to diet composition (131).

The effects of micronutrients on IGFBP-1 production has received limited attention. Treatment of male rats with sodium selenite led to growth retardation with apparent reduced levels of serum IGFBP-1 and/or IGFBP-2 and IGFBP-3 concentrations, as determined by ligand blot, and serum IGF-I measured by immunoassay (132). Serum insulin concentrations were unaffected.

Growth Hormone. A direct effect of GH on IGFBP-1 expression *in vitro* has been controversial. In rats, hypophysectomy leads to increased levels of hepatic IGFBP-1 gene transcription and mRNA. In addition, increased serum IGFBP-1 concentrations have been observed in genetic GH deficiency in rats (133) and GH therapy results in a fall in hepatic IGFBP-1 mRNA which is apparently mediated at the level of transcription since a single GH dose returned the gene transcription rate and mRNA to control levels within 30 and 60 min, respectively (4). Furthermore, chronic GH treatment of genetically GH deficient *dw/dw* rats leads to a fall in IGFBP-1 concentrations (134). However, the possibility that the GH effects in these *in vivo* studies were mediated by GH-induced changes in other hormones, such as insulin, must be considered, and would be consistent with the observation that serum IGFBP-1 levels are not different in genetically GH-deficient *lit/lit* mice as compared with their heterozygous litter mates (135). Moreover, GH had no effect on rat hepatocytes cultured in the presence of insulin, or in rat and human hepatoma cells (87, 88, 121, 136–138).

On the other hand, a small effect of GH to decrease IGFBP-1 mRNA abundance has been reported in isolated rat hepatocytes cultured in serum-free media (124, 139, 140). Cultured rat hepatocytes transfected with IGFBP-1/chloramphenicol acetyltransferase reporter plasmids responded to exogenous GH with transient suppression of IGFBP-1 promoter activity and GH, but not insulin, was able to inhibit IGFBP-1 expression in hepatocytes from hypophysectomized rats (141). However, the maximal suppressive effect of GH was quite modest compared with that of insulin. GH inhibition of IGFBP-1 expression in primary rat hepatocytes appears to involve activation of protein kinase C pathways (139, 140), and it has been postulated that the region of the rat IGFBP-1 promoter between 277 and 930 bp 5′ to the transcription start site may confer the GH effect (141). These and other data (141, 142) suggest that GH could play a role in modulating the dominant insulin regulation of IGFBP-1 transcription in rats. Similar findings have not been reported in human studies, and it has been suggested that species differences in the GH-insulin axis in human versus rat may distinguish the regulation of IGFBP-1 (141).

An inverse relationship between serum IGFBP-1 and insulin has been observed in GH-deficient patients during eu- and hypo-glycemic clamp studies (111) and during GH treatment (143–145), and in acromegalic patients before and after adenectomy (112), which is consistent with our previous report that GH and glucose do not have independent effects on the acute regulation of serum IGFBP-1 (106). No differences in IGFBP-1 patterns were observed between GH-deficient patients treated with either subcutaneous infusion or daily injections of GH (146). IGFBP-1 levels are also reported to be inversely correlated with total IGF-I in acromegaly (77). In acromegalic patients, treatment with octreotide or bromocriptine both led to decreased serum GH levels; however, only octreotide was associated with increased serum IGFBP-1 concentrations, arguing against a role for GH (147).

The possibility that chronic GH status might affect the level of IGFBP-1 responsiveness to insulin has been suggested by two recent studies. Hilding *et al.* (148) found that serum IGBP-1 levels were elevated in relation to insulin in GH deficient adults as compared with healthy controls, although no significant long-term effects of GH replacement therapy on serum insulin or IGFBP-1 concentrations were observed (149). Lee *et al.* (149a) recently reported pretreatment and GH treatment studies in children and young adults with GH deficiency. Although IGFBP-1 levels did not change significantly during GH treatment as compared with pretreatment, insulin levels showed a marked increase, re-

sulting in a decreased ratio of IGFBP-1 to insulin during GH treatment. These data suggest that GH, perhaps by affecting IGF production, may affect tonic hepatic responsiveness of IGFBP-1 to insulin. In this respect, it is interesting to note the structural and possible functional similarities between the GH receptor and cytokine receptors (150), since cytokines are also postulated to mediate a resistance of hepatic IGFBP-1 production to insulin (*vide infra*).

In contrast to these data in GH-deficient individuals, GH treatment of pediatric patients with Turner's syndrome (151) or chronic renal failure (152) results in decreased serum IGFBP-1 concentrations associated with a rise in serum insulin levels. This discrepancy could be related to the fact that these conditions are associated with normal or elevated endogenous serum GH levels; however, the possible contributory mechanisms are obscure.

Growth hormone treatment of normal lactating women for 10 days caused a slight decrease in both plasma and milk IGFBP-1 concentrations; milk IGFBP-1 levels were 10%–20% of plasma concentrations (153).

IGF-I and IGF-II. IGF-I and II inhibit IGFBP-1 expression in HepG2 cells and in luteinized human granulosa cells which possess abundant type I IGF receptors (85, 154–156), but not in normal adult rat and human liver cells where type I IGF receptors are low or absent (87, 137). In the responsive systems, the IGFBP-1–inhibitory potencies of IGF-I and IGF-II are equal to or greater than that of insulin. Furthermore, des(1,3) IGF-I, which has low affinity for IGFBPs but normal type I IGF receptor-binding affinity, was 10-fold more potent than insulin (155). In HepG2 cells, both IGF-I and IGF-II, apparently acting through the type I IGF receptor, inhibit IGFBP-1 mRNA levels and promoter activity in a dose-dependent manner, suggesting similarities with the mechanism by which insulin inhibits gene expression (154).

Suppression of serum IGFBP-1 concentrations has been reported both in normal and diabetic subjects following IGF-I administration (157). However, other studies have demonstrated increased serum IGFBP-1 concentrations following subcutaneous injection of IGF-I (158–162); in some cases this appeared to be related to acute suppression of insulin secretion. Moreover, intravenous infusion of IGF-I following colorectal surgery had no effect on serum IGFBP-1 levels (163). No significant effects of IGF-I treatment on IGFBP-1 have been reported in patients with growth hormone insensitivity (164–166). In all of these situations, the effects of exogenous IGF-I are likely to be related to a complex balance between acute changes in free IGF-I and acute and chronic changes in insulin secretion and discrepant results between studies are probably due to differences in experimental conditions.

Glucagon and cAMP. Glucagon stimulates IGFBP-1 production by human fetal liver explants and primary rat hepatocyte cultures (86, 137, 167). In primary cultures of rat hepatocytes, the stimulatory effect of glucagon peaks at 3 hr and returns to baseline by 12 hr and glucagon was as potent as dexamethasone in increasing IGFBP-1 mRNA levels (4, 139, 140). In contrast, no effect of glucagon on IGFBP-1 production was seen in other studies with primary cultures of rat hepatocytes (87, 121). In some of these latter experiments, the use of low concentrations of insulin to optimize viability of primary hepatocytes *in vitro* may have blocked the glucagon effects, since the IGFBP-1 inhibitory effects of insulin are dominant over the stimulatory effects of glucagon (167), and the low levels of insulin may have precluded observation of a stimulatory effect. A possible mechanism for IGFBP-1 stimulation by glucagon *in vitro* is suggested by the observation of glucagon inhibition of IGF-I production by primary rat hepatocytes (121).

Alternatively, since many metabolic actions of glucagon are mediated by cyclic adenosine monophosphate (cAMP), it is reasonable to postulate that cAMP acts as a second messenger to mediate the stimulatory effects of glucagon on hepatic IGFBP-1 expression *in vitro*. Consistent with this hypothesis, cAMP and theophylline (which increases intracellular cAMP levels) both stimulate IGFBP-1 protein and/or mRNA in human fetal liver explants, primary hepatocytes, and hepatoma cells. In HepG2 (human) and H4IIE (rat) hepatoma cells, this effect is apparently mediated at the level of gene transcription since both cAMP and theophylline have been reported to directly stimulate IGFBP-1 promoter activity (47, 53, 59, 168). In HepG2 cells, this effect is conferred, in part, through a cAMP response element (CRE) located at −293 to −249 bp 5′ to the hIGFBP-1 transcription start site, a region which binds CRE-binding protein (58). This CRE is not conserved in the rat promoter and elements conferring cAMP response in the rat IGFBP-1 promoter are not yet definitively mapped (53). In decidual endometrial cells, cAMP stimulates IGFBP-1 directly and mediates the IGFBP-1 stimulatory effects of relaxin (4).

Overall, a stimulatory effect of glucagon on IGFBP-1 expression seems well supported by the *in vitro* data, and this effect could be mediated by cAMP and/or glucagon effects on IGF-I production. However, *in vivo* studies have shown variable effects of glucagon on IGFBP-1 (4, 114, 169) and the physiologic relevance of these *in vitro* observations is uncertain.

Steroids. *In vitro* and *in vivo* investigations have been consistent in confirming insulin and glucocorticoid as the major physiologic regulators of hepatic IGFBP-1 expression (4, 87, 107, 121, 137, 139, 140, 170, 171). Glucocorticoid is stimulatory and insulin is inhibitory for both basal and glucocorticoid-stimulated IGFBP-1 gene transcription. However, *in vivo* stimulatory effects of glucocorticoid have thus far been observed only with concomitant hypoinsulinemia in humans (107) and rats (172), which is consistent with the predominant suppressive effect of insulin characterized *in vitro* (59). In rat H4IIE hepatoma cells, the stimulatory effects of glucocorticoid were inhibited by the progesterone receptor antagonist, RU486 (170).

Glucocorticoids appear to stimulate hepatic hIGFBP-1 gene transcription through elements in the proximal promoter. GRE1, spanning bp −193 to −179, and GRE2, bp −102 to −88, are poorly conserved GREs which nevertheless bind glucocorticoid receptor (GR). Both GREs must be functional for dexamethasone to stimulate hIGFBP-1 promoter activity in HepG2 cells (57). In addition to these GREs, an intact IRE is essential for maximal stimulation of hIGFBP-1 promoter activity by dexamethasone (57). This is reminiscent of the glucocorticoid response unit described in the PEPCK promoter, which requires two poorly conserved GREs and an IRE for maximal glucocorticoid stimulation (173). Similarly, the TAT promoter also requires an IRE for maximal glucocorticoid stimulation (174). Recent studies, which show that binding of HNF3 proteins to IRE mutants correlates with responsiveness of these mutants to glucocorticoids (albeit not insulin, *vide supra*), provide indirect evidence that an HNF3 form(s) is the IRE-binding factor which confers maximal glucocorticoid stimulation of IGFBP-1 and PEPCK promoter activities in hepatocytes (60, 97, 175).

In contrast to the hIGFBP-1 promoter, the rat promoter appears to have only one functional GRE which binds GR (52). As with the human GREs, the rat GRE requires the IRE and other promoter elements for maximal stimulation of promoter activity by glucocorticoids in hepatocytes (52, 176, 177, 178).

Recent studies demonstrate that glucocorticoids stimulate IGFBP-1 protein and mRNA expression in cultured primary human osteoblasts, and that insulin potently inhibits this stimulation (91), suggesting the possibility that the mechanisms of IGFBP-1 gene regulation by glucocorticoids and insulin are identical in hepatocytes and osteoblasts. Corollary data in other tissues has not yet been reported.

Although adrenal and gonadal sex steroids may affect local tissue production of IGFBP-1, an effect on serum IGFBP-1 concentrations has not been convincingly demonstrated. Serum IGFBP-1 levels were correlated with sex hormone binding globulin (SHBG) and the testosterone/SHBG ratio and negatively correlated with IGF-I in postmenopausal women with breast cancer without endocrine treatment (179). However, serum IGFBP-1 levels were unrelated to serum estradiol, estrone or androstenedione levels. Replacement doses of dehydroepiandrosterone in elderly men and women led to decreased serum IGFBP-1 and increased IGF-I concentrations with no apparent change in insulin sensitivity (180). In addition, oxandrolone augmented the decrease in IGFBP-1 induced by GH treatment in Turner's syndrome (151). Other relationships between IGFBP-1 and gonadal steroids are discussed in later sections.

Thyroid Hormones. IGFBP-1 is probably not expressed in thyrocytes (181), although protein immunostaining showed diffuse distribution of IGFBP-1 in normal and malignant thyroid (182).

In rats, hypothyroidism is associated with increased hepatic expression of IGFBP-1 mRNA, while IGFBP-1 mRNA levels are normal in hyperthyroidism (183). Somewhat divergent results have been observed in human studies. A placebo-controlled, crossover trial in 12 patients with hypothyroidism demonstrated an average 2-fold increase in fasting serum IGFBP-1 concentrations within 3 months of thyroxine treatment (184). There were no changes in serum IGF-I concentrations and insulin levels were not measured. Conversely, serum IGFBP-1 levels decreased with thyroxine withdrawal in hypothyroid patients (185, 186). This change was associated with decreased IGF levels in both studies. IGFBP-1 levels were elevated in patients with hyperthyroidism and decreased during treatment (187).

These data are consistent with *in vitro* studies in which tri-iodothyronine (T3) treatment of human HepG2 hepatoma cells caused a dose-dependent increase in IGFBP-1 concentrations in conditioned medium within 24–48 hr of exposure accompanied by increased IGFBP-1 mRNA concentrations (41). Treatment with cycloheximide, an inhibitor of protein synthesis, caused an early (3–12 hr) rise in IGFBP-1 mRNA concentrations, perhaps due to prolongation of IGFBP-1 mRNA $T_{1/2}$, followed by inhibition of the stimulatory effect of T3 on IGFBP-1 mRNA. These latter results indicate that thyroid hormones may act by affecting synthesis of a protein which modulates IGFBP-1 synthesis.

Somatostatin. The long-acting somatostatin analog octreotide had a delayed effect to increase IGFBP-1 mRNA in HepG2 cells (188) and to increase hepatic IGFBP-1 mRNA within 1 hr of administration in rats (189). Octreotide is known to inhibit expression of IGF-I (190–192) as well as insulin, GH, and other hormones. Therefore, this observation may reflect either a direct stimulatory effect of octreotide on IGFBP-1 expression or an indirect effect via inhibition of IGF secretion by the strain of HepG2 cells studied (not all strains of HepG2 cells secrete IGFs).

In vivo data suggest that octreotide has no significant independent effect on IGFBP-1 levels, and that the increased serum IGFBP-1 levels are due to octreotide inhibition of insulin secretion (193). Administration of octreotide to women with polycystic ovary syndrome, who have high serum insulin and low serum IGFBP-1 concentrations, led to decreased insulin and increased IGFBP-1 levels within 60 min (194). Administration of two doses of lanreotide, a somatostatin analog, resulted in a delayed dose-related increase in serum IGFBP-1 without a dose-related change in serum insulin concentrations (195); however, hepatic portal insulin concentrations could not be assessed in this study. In healthy volunteers studied with a eu-glycemic, basal insulinemic clamp, infusion of native somatostatin-14 led to a delayed increase in serum IGFBP-1 concentrations; this effect was not observed during euglycemic, hyperinsulinemic clamp studies (196). Although the investigators postulated an independent stimulatory action of somatostatin, mediation by decreased hepatic/portal concentrations of IGF-I and/or endogenous insulin could not be excluded. In addition, somatostatin has recently been shown to decrease

peripheral insulin clearance (197); therefore, changes in serum insulin concentrations may not accurately reflect acute changes in hepatic portal insulin levels.

Epidermal Growth Factor. Epidermal growth factor, which is believed to play a role in liver regeneration, increases IGFBP-1 synthesis in human hepatoma cells (198) and is postulated to regulate IGFBP-1 production in the DU145 human prostate cancer cell line (199). Furthermore, EGF administration in newborn rat pups causes acute increases in both hepatic IGFBP-1 mRNA and serum IGFBP-1 levels which were apparently unrelated to change in serum insulin concentrations (200). Serum IGFBP-1, as assessed by ligand blot, increased during 4 weeks of EGF treatment in minipigs while insulin levels did not change (201). Triiodothyronine levels increased and IGF-I decreased in this latter study, indicating possible mechanisms for the changes in IGFBP-1. Therefore, IGFBP-1 could play an *in vivo* role to mediate or augment the somatic growth inhibitory effects of EGF. EGF had no effect on the polarity of IGFBP-1 secretion from Caco-2 human intestinal epithelial cells (202); the relevance of this finding is uncertain since IGFBP-1 secretion has not been identified in normal human intestinal tissue.

Protein Kinase C. We previously demonstrated that both phorbol esters and inhibitors of protein kinase C (PKC) caused dramatic stimulation IGFBP-1 production in human HepG2 hepatoma cells, and that these effects could suppress the inhibitory actions of insulin (203). Based on our data, we postulated that IGFBP-1 expression is upregulated by downregulation of PKC—that is, the stimulatory effects of phorbol esters on IGFBP-1 expression are due to downregulation of PKC. Limited data also indicated that phorbol esters did not affect the degradation rate of IGFBP-1 mRNA in these cells (Lee and Snuggs, unpublished data). Similar stimulatory effects of phorbol esters on IGFBP-1 expression (4) have been reported in other cell types, including a recent report in cultured human fetal adrenal cells (204). (The latter study is countered by a recent report that failed to identify IGFBP-1 mRNA in fetal rhesus monkey adrenal [205].) Our results in human cells differ somewhat from studies in rat hepatoma cells in which the stimulatory effects of phorbol esters are of relatively short duration. Lewitt *et al.* (170) postulated that this species discrepancy might be due to the presence of a regulatory element in the human IGFBP-1 promoter which is similar to the phorbol ester/insulin negative-response element identified in the PEPCK gene. However, our unpublished data in human hepatoma cells do not support this hypothesis, since phorbol esters had no effect on the activity of IGFBP-1 promoter constructs containing the IRE. Although the data are consistent with our previously stated hypothesis that the PKC system may be involved in tonic regulation of IGFBP-1 synthesis (4, 203), further studies are clearly needed to define the physiologic relationships between the PKC system and IGFBP-1.

Cytokines. Cytokines are a diverse group of factors which, among other actions, mediate the inflammatory response. Recent data suggest that cytokines may also regulate IGFBP-1 production. Interleukin-1β (IL-1β) inhibits production of IGFBP-1 by decidualized endometrial stromal cells *in vitro* (206) (*vide infra*). Conversely, *in vivo* infusion of IL-1β or TNFα stimulates hepatic production of IGFBP-1 in rats (207–209). Recently, Samstein *et al.* (116) reported that, in addition to their *in vivo* IGFBP-1–stimulatory effects, IL-1β, TNFα, and interleukin-6 (IL-6) all stimulated production of IGFBP-1 by HepG2 human hepatoma cells. In addition, the stimulatory effect of IL-6, but not of the other cytokines, was dominant over the inhibitory action of insulin. These results could help to explain the paradoxical increase in IGFBP-1 which occurs during trauma, surgery, and infection, as discussed in later sections.

General Regulatory Actions of IGFBP-1

The vast majority of *in vitro* studies support a role for IGFBP-1 in inhibiting IGF-stimulated growth and differentiative function. Thus, addition of IGFBP-1 to a variety of cell systems has been shown to attenuate exogenous and endogenous IGF action (4, 210–218). This is not surprising since IGFBP-1 can bind to IGFs with high affinity and prevent their activation of receptor signalling. *In vivo* studies are also consistent with an inhibitory role of IGFBP-1 on IGF action (219–222).

Brain growth is inhibited in IGFBP-1 transgenic mice overexpressing IGFBP-1 in brain tissue (42, 43, 44, 222, 223). In mice overexpressing the hIGFBP-1 cDNA under the control of the metallothionein promoter, IGFBP-1 expression was not associated with abnormalities of growth or glucose metabolism (42, 44). However, in mice overexpressing the rat IGFBP-1 chromosomal gene under the control of the phosphoglycerate kinase promoter, IGFBP-1 expression was associated with impaired somatic growth and hyperglycemia (222, 224). These experimental differences may be the result of differences in serum IGFBP-1 levels, the relative expression levels of the human versus rat IGFBP-1 transgenes, or to use of different promoters leading to different patterns of tissue-specific IGFBP-1 expression. Nevertheless, it seems clear that IGFBP-1 overexpression is associated with evidence of impaired IGF action and that enhanced IGF action is not observed.

Conversely, a few studies indicate that under some circumstances IGFBP-1 may enhance the mitogenic actions of IGF (225–230). However, a specific set of requirements appear to be necessary for observation of these effects. In general, a full potentiation effect required low concentrations of IGFBP-1, IGF-I binding to IGFBP-1, IGF-I binding to the type I IGF receptor, and the presence of a heat and acid stabile factor(s) in platelet-poor plasma.

The serum half-life ($t_{1/2}$) of IGFBP-1 has been estimated in several studies (4) and is generally in the range of 7–13 min. In rat studies, the $t_{1/2}$ of exogenously administered IGFBP-1 is apparently unaffected by co-

administration IGF-I; however, IGFBP-1 increases the $t_{1/2}$ of IGF-I from a mean of 1.2 min to 5.3 min (220).

The role of IGFBP-1 phosphorylation in regulating IGFBP-1 activity is controversial. As discussed in a preceding section, *in vitro* studies in which specific serine phosphorylation sites are mutated result in three to five lower affinity of IGFBP-1 for IGF-I. A similar decrease in affinity is observed for purified nonphosphorylated versus phosphorylated human amniotic fluid IGFBP-1 (231). This has led to the widely accepted hypothesis that phosphorylation might have a regulatory role in modulating the effect of IGFBP-1 in IGF action. However, it should be noted that the 3- to 5-fold lowered affinity of the nonphosphorylated form still reflects a relatively high affinity of IGFBP-1 for IGFs; for instance, Koistinen (231, 232) reported K_a values of 1.2 and 3.1–4.6 $\times$ 10^{-9} for nonphosphorylated and phosphorylated amniotic fluid IGFBP-1, respectively. Although discrepant biological effects of non- and phospho-isoforms of IGFBP-1 have been reported in some experimental systems (233), this has not been a consistent observation. Purified nonphosphorylated and phosphorylated IGFBP-1 isoforms from human amniotic fluid both blocked IGF-I binding to human fetal fibroblasts and, through undefined mechanisms, enhanced IGF-I-mediated thymidine incorporation (IGFBP-1 alone had no effect) (231). Nonphosphorylated IGFBP-1 has been shown to potently inhibit IGF action and cell growth both *in vitro* (234–236) and *in vivo* (215, 221). Perhaps most importantly, physiologic regulation of IGFBP-1 phosphorylation has not been demonstrated, and virtually all of the IGFBP-1 present in nonpregnant human serum is highly phosphorylated. Overall, it is uncertain whether phosphorylation plays an *in vivo* physiologic role in the regulation of IGFBP-1 action.

IGFBP-1 contains an Arg-Gly-Asp consensus sequence for cell attachment, and it has been suggested that cell surface association is necessary for IGFBP-1 to potentiate IGF action (237). This phenomenon is discussed further in other sections.

Clinical Physiology and Pathophysiology

Liver and Reticuloendothelial System. Liver is the primary source of serum IGFBP-1 in males and in nonpregnant females. Within the liver, IGFBP-1 production is localized to the hepatocytes, in contrast to IGFBP-3, which has been localized to the Kupffer cells (4, 136, 238, 239). Hepatocyte expression of IGFBP-1 is upregulated in fetal development and in liver regeneration and parallels expression of other genes associated with liver regeneration (5, 240, 241). In rats following partial hepatectomy, the increase in hepatic IGFBP-1 mRNA has been variably reported to be accompanied by increased (240) or unchanged (242) serum levels of a small MW IGFBP identified as IGFBP-1 by ligand blot. Increased IGFBP-1 mRNA has also been reported in livers from protein-restricted rats (130).

Fasting serum IGFBP-1 levels are elevated in patients with cirrhosis and in normal adults following ethanol ingestion despite increased C-peptide or insulin concentrations (4, 243–245). Although fasting IGFBP-1 and C-peptide levels were not correlated in cirrhotic patients, an acute fall in IGFBP-1 levels was noted during oral glucose tolerance testing (244). In this same population, GH levels were elevated while IGF-I and IGFBP-3 levels were low, probably reflecting hepatic GH resistance. The net result of these changes could theoretically result in a lowered fraction of free IGF-I. Increased serum levels of IGFBP-1 in cirrhosis are apparently due to increased hepatic production, rather than to changes in renal clearance (246). Increased IGFBP-1 levels in cirrhosis could, in part, be related to increased cytokine levels (116) (*vide infra*).

IGFBP-1 mRNA was not detected in rat spleen under conditions of hypophysectomy, GH-treatment, or IGF-I treatment (247). In addition, IGFBP-1 was not found in normal or stimulated human lymphocytes (248).

Female Reproductive System. *Nonpregnant uterus.* The uterine endometrium is a dynamic tissue that undergoes cyclic changes in response to circulating ovarian steroids (249); many of these responses are mediated by growth factors and related peptides (250, 251). During the first half (estrogen-dominant, proliferative phase) of the menstrual cycle, endometrial cells proliferate. After ovulation and under the influence of progesterone (secretory phase), cellular differentiation predominates, heralded by unique secretions from the glands and stromal decidualization. In the absence of blastocyst implantation, the hypertrophied uterine endometrial tissue is shed with the menses and the cycle is renewed.

Interest in the IGF system in relation to uterine endometrium arose from two landmark findings. The first was the report of high levels and marked estradiol (E_2) dependence of IGF-I mRNA in rat uterus (252). The second was the discovery that one of the major secretory proteins of decidualized endometrium was an IGF-binding protein, IGFBP-1 (253–255). The IGF system is one of several growth factor systems that are important in endometrial cyclic development and blastocyst implantation (250, 256). In addition, IGF-I is postulated to be an estrogen mediator, or estromedin, in the pathogenesis of endometrial cancer (257) (*vide infra*).

Components of the IGF system in human endometrium undergo unique cyclic changes throughout the menstrual cycle (Table I). In rat uterus, IGFBP-1 mRNA levels are reported to peak during diestrus and reach a nadir during proestrus (265), implying that estrogen may downregulate IGFBP-1 expression. However, other investigators have reported that IGFBP-1 mRNA is not detectable in rat uterus during the estrous cycle (266).

Cell-specific expression of mRNAs encoding the IGFs and their receptors indicate potential autocrine (in the stroma) and paracrine (in the stroma and epithelium) roles for the IGFs in endometrial cell function. Supporting this are the findings that IGF-I and IGF-II are mitogenic for

Table I. Relative Expression of IGF Family mRNAs in Human Endometrium, Early Pregnancy Decidua, and Trophoblast

	IGF-I	IGF-II	IGFBP-1	IGFBP-2	IGFBP-3	IGFBP-4	IGFBP-5	IGFBP-6	IR*	IIR*
Proliferative Phase										
Epithelium	–	–	–	–	–	–	–	–	+++	++
Stroma	++++	++	–	+	+	+	++++	+	+	++
Secretory Phase										
Epithelium	–	–	–	–	–	–	–	–	+++	++
Stroma	+	++++	++++	++	++	+	–	++	+	+++
Decidua	–	–	++++++	++	++	+	±	+++	++	++
Trophoblast	–	+++++	–	–	+	–	–	–	+++	+++

Note. Relative expression is graded from none (–) to maximal (++++++) based on published data (250, 254, 258–264).
[a] IR, type I IGF receptor; IIR, type II IGF receptor.

cultured endometrial cells and are related to regular secretory functions of stromal cells (267, 268).

Messenger RNAs encoding the IGFBPs 1 through 6 (Table I) are expressed in human endometrium, primarily in stroma (258–262, 267). IGFBP-1, 2, 3, and 6 mRNAs are expressed differentially in the secretory phase, with IGFBP-1 in highest abundance. While IGFBP-1 expression is limited primarily to the stromal cells, scattered expression in glandular epithelium has been reported (258). In contrast, IGFBP-1 is not expressed in the uterine myometrium (270–272) or in leiomyomata (273). The temporal and spatial relationships of IGF and IGFBP-1 expression in the endometrium suggest that IGFBP-1 plays a major role in regulating IGF availability to receptors on both glandular epithelium and stroma.

The production and regulation of IGFBP-1 in the uterine endometrium has been a subject of considerable recent study. In humans, IGFBP-1, also previously known as placental protein-12 (274) and α_1-progesterone–dependent endometrial globulin (α_1-PEG) (255), is a major product of late secretory endometrium (16 mcg/g tissue protein) and pregnancy decidua (1224 mcg/g) (275–278). Explant cultures of secretory endometrium and early pregnancy decidua also secrete large amounts of IGFBP-1 *in vitro* (275, 276, 278).

Human endometrial stromal cells, which undergo *in vivo* proliferation and differentiation in response to progesterone can be decidualized *in vitro* with progesterone or progestins relaxin, estradiol, epidermal growth factor (EGF) (279–283); the progestin effect can be augmented by cAMP (284) or free α-subunit (285). In these cells, IGFBP production patterns are dependent on the degree of decidualization (267, 269, 280). Within the endometrium *in vivo,* IGFBP-1 mRNA is primarily expressed in decidualized endometrial stroma; therefore, the *in vitro* model of decidualization has provided an opportunity to investigate production and regulation of IGFBP-1. In nonhuman primates, endogenous estrogen and progesterone act synergistically to increase endometrial IGFBP-1 levels, and progesterone is important for maximal IGFBP-1 expression (286). Changes in the complement of IGFBPs secreted by endometrial stromal cells upon decidualization *in vitro* and the cycle dependence of most IGFBP mRNAs in endometrium *in vivo* (258, 259, 261) suggest that they are regulated by steroid hormones. Without progesterone, endometrial stromal cells synthesize and secrete very low levels of IGFBP-2, 3, and 4, and no detectable IGFBP-1. However, with progesterone, very high levels of IGFBP-1 are produced upon decidualization (25.3 ± 3.2 mcg/day/10^6 cells) in contrast to another decidual marker, prolactin (40 ng/day/10^6 cells) (267, 282, 287) and this action is inhibited by the progesterone antagonist, RU486 (282, 287). Lane *et al.* (288) reported even more dramatic results in endometrial cells cultured in the presence of medroxyprogesterone acetate and relaxin for 20 days; prolactin levels increased from 0.004 to 7 mcg/day/10^6 cells and IGFBP-1 increased from 0.01 to 44 mcg/day/10^6 cells.

IGF-I and IGF-II play roles in the microenvironment of the decidualized endometrium, where there are potential autocrine/paracrine regulatory mechanisms that may influence endometrial stromal IGFBP production and, therefore, stromal and glandular function and trophoblast-stromal interactions. In endometrium, insulin and the IGFs inhibit decidualizing stromal cell IGFBP-1 secretion into conditioned media (267) in a dose-dependent fashion, with ED_{50} concentrations which are consistent with action through their respective receptors (267). IGFs are also inhibitory to IGFBP-1 production by endometrial cells decidualized *in vivo* (289). In contrast, relaxin, which is structurally homologous with the IGFs, stimulates IGFBP-1 production by decidualized endometrial cells *in vitro* (282). The physiologic relevance of IGF and insulin regulation of IGFBP-1 likely rests in the need to regulate IGF bio-availability to target tissues, including trophoblast and/or decidua and glandular epithelium in pregnant and nonpregnant endometrium. In addition, these regulatory mechanisms may affect direct interactions of IGFBP-1 with the invading trophoblast (*vide infra*).

In vitro and *in vivo* studies support modulatory roles for IGFBP-1 in endometrial cell function. For example, IGFBP-1 inhibits IGF binding to the endometrial membrane (290) and inhibits the mitogenic effects of IGF-I on endometrial stroma *in vitro* (268). IGF-I is postulated to be an estromedin, mediating the mitogenic effects of E_2 on endo-

metrial cells (252), and E_2 has an inhibitory effect on uterine IGFBP-1 expression, as demonstrated in the rodent uterus (291). Therefore, E_2-induced uterine endometrial proliferation involves both enhanced expression of estromedins, including IGF-I, and attenuation of IGF inhibitors (i.e., IGFBP-1). Recently, an IGFBP-1 transgenic mouse model was used to further explore this relationship *in vivo*. In this system, IGFBP-1 was expressed in the uterine glandular epithelium and impaired ability of E_2 to stimulate DNA synthesis was observed compared with wild-type controls (292). These observations support the hypothesis that IGFBP-1 inhibits endometrial E_2 action, probably *via* inhibition of IGF-I action.

Cancers infrequently occur in the uterine endometrium, but when they do they are generally estrogen dependent. In humans, there is strong evidence for a role of IGFs in the pathogenesis of endometrial hyperplasia and cancer, both of which are usually E_2-dependent disorders (257, 293). IGFBP-1, induced by progesterone, is believed to be protective against IGF-I and, therefore, E_2, an effect which would theoretically be associated with endometrial glandular atrophy and minimization of hyperplastic and neoplastic processes (257). Alternatively, the risks of endometrial hyperplasia and neoplasia are increased in anovulation and hyperinsulinemia, disorders in which IGFBP-1 levels are suppressed and, theoretically, the protective effects of IGFBP-1 are diminished. A model for the protective effects of IGFBP-1 in endometrium is shown in (Fig. 3).

The possibility of testing this hypothesis is suggested by experience with the progestin IUD. In studies involving up to 5 years of exposure, the progestin IUD, but not subcutaneous progestin implants or copper IUDs, has a striking effect to increase endometrial IGFBP-1 production, although no apparent effect on serum IGFBP-1 is observed (294, 295). In postmenopausal women receiving estrogen replacement therapy, stromal decidualization with increased IGFBP-1 production and epithelial atrophy are associated with the progestin IUD but not with subcutaneous progestin implants (296). The effects of the progestin IUD to induce endometrial IGFBP-1 production may offer a means of protection against hyperplasia and neoplasia, especially in disorders with abnormal cyclic production of progesterone (e.g., oligo- and an-ovulation) (257).

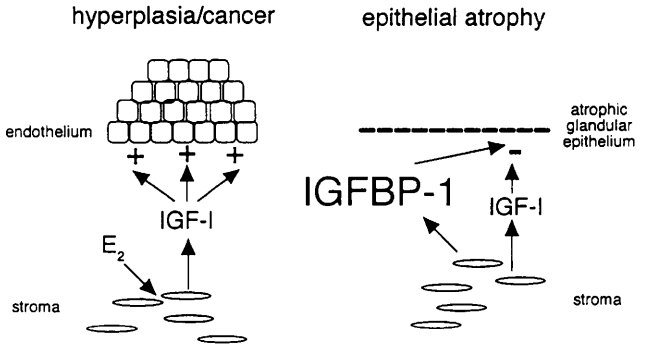

Figure 3. IGFBP-1 as an antiestrogen in human endometrium (see text for details).

In these studies, the lack of change in serum IGFBP-1 levels with changes in the status of endometrial differentiation is not surprising given the much greater hepatic contribution to the serum IGFBP-1 pool. Similarly, administration of an antiprogestin was associated with histologic evidence of inhibited endometrial decidualization although serum IGFBP-1 levels did not change (297).

IGFBP-1 and other components of the IGF system have been identified in peritoneal fluid from normally cycling women (298) and could play a role in the pathogenesis of endometriosis. IGFBP-1 concentrations in this fluid were similar to serum levels. In a study of breast cancer patients, tamoxifen treatment was associated with a ~2-fold increase in nonfasting, serum IGFBP-1 levels, although considerable overlap was noted with the tamoxifen-untreated group (299). Within the treated group, those with proliferative uterine endometrial epithelium had higher IGFBP-1 than those with atrophic epithelium. Finally, IGFBP-1 may be absorbed into the peripheral circulation with irrigation fluids during endometrial resection, and could serve as a marker for leakage of endometrial products during endometrial surgical procedures (300).

Implantation and pregnancy. In addition to physiologic roles during the menstrual cycle, the IGFs and IGFBPs may be major factors in blastocyst implantation. The abundance of IGFBP-1 in the maternal endometrium of pregnancy (decidua) and the exclusive expression of IGF-II in the trophoblast are highly suggestive of a regulatory role for IGFBP-1, either as an IGF-binding protein or *via* IGF-independent effects at the maternal-fetal interface (263).

In monkeys and humans IGFBP-1 mRNA and protein are highly expressed in decidua (264, 301). In addition, it is present at or near the implantation site in cat decidua (302) and at the deciduo-trophoblastic interface in human early pregnancy specimens (303). This is in contrast to the situation in rodents, in which IGFBP-1 appears to be localized to the glandular epithelium and is not specifically associated with decidualization (304, 305). In baboons, endometrial IGFBP-1 mRNA and protein are responsive to estrogen and progesterone, are only detectable in the secretory phase, and are maximal in decidualized endometrium (286, 306, 307). Unlike in humans, however, IGFBP-1 expression is in the epithelial cells of the deep basal glands during the secretory phase (308). During pregnancy, the site of epithelial IGFBP-1 expression moves from the deeper glands to the stroma. Using the simulated pregnant baboon model (309), it has been shown that the conceptus is a prerequisite for the complete morphological and biochemical transformation of a stromal fibroblast to a decidual cell, replete with IGFBP-1 synthesis (310, 311). This is in contrast to the human endometrium in which decidualization occurs even in the absence of a conceptus, although to a greater degree during pregnancy. IGFBP-1 immunoreactivity has been localized to the extracellular matrix and stromal cells of the decidu-

alizing endometrium, in the periarteriolar regions, and in the villous trophoblast, but not on placental fibroblasts (255, 263, 312–315).

On the other hand, the placental cytotrophoblast expresses high levels of IGF-II mRNA (264, 301, 316), as well as other cytokines and growth factors. During conception, the close proximity of the invading trophoblast and the decidua prompts the question of whether growth factors and cytokines present at this interface regulate decidual IGFBP-1 production. A recent study has investigated the effects of the IGFs, interleukin-1β (IL-1β), transforming growth factor–β (TGFβ), stem cell factor (SCF), colony-stimulating factor–1 (CSF-1), leukemia-inhibitory factor (LIF), and IGF-II on decidualized endometrial stromal IGFBP-1 production (206). IGF-II and IL-1β were the only factors that had effects on IGFBP-1 levels and both were inhibitory with ED_{50} concentrations within physiologic ranges. IL-1β has also been reported to inhibit the process of decidualization of endometrial stromal cells (317, 318).

Another trophoblast product, human chorionic gonadotropin (hCG), has been reported to stimulate stromal IGFBP-1 production, probably due to its promotion of stromal decidualization (319, 320). Similar results have been reported with free hCG α subunit (285), an action which occurs through undefined mechanisms and is presumably not specific for hCG since the α subunit structure is shared with other hormones. On the other hand, hCG has no apparent effect on stromal cell products following decidualization *in vivo* (321), and IGFBP-3, but not IGFBP-1, blocks the stimulatory actions of hCG in isolated, perfused rabbit ovaries (322).

During the invasive phase of implantation, the "intermediate" trophoblast of the anchoring villous, producing large amounts of IGF-II, invades the maternal decidual stroma which is producing large amounts of IGFBP-1 (264, 323) (Fig. 4). Trophoblast invasion into the maternal endometrium occurs by processes which are similar to those accompanying tumor invasion, including (i) attachment to the extracellular matrix, (ii) local matrix proteolysis, (iii) cell migration, and (iv) inhibition of these actions (324). IGF-II and IGFBP-1 are temporally and spatially positioned to participate in the regulation of these processes. In addition, IGFBP-1 is relatively resistant to proteolysis (10, 11, 260, 325–329), which is an important process during decidualization and implantation (330). For IGF-II, a gradient of mRNA abundance is found in the trophoblast columns with the greatest levels expressed at the invading front, suggesting an active role for IGF-II in trophoblast invasion. IGFBP-1 has been shown to have inhibitory effects on IGF-binding and IGF-mediated mitogenesis in choriocarcinoma cells *in vitro* (331); a similar relationship between IGF-II and IGFBP-1 at the trophoblast/decidua interface is conceivable. However, precise mechanisms for IGF-II action and interactions with IGFBP-1 during trophoblast invasion have not yet been defined.

IGFBP-1 also may have IGF-independent actions me-

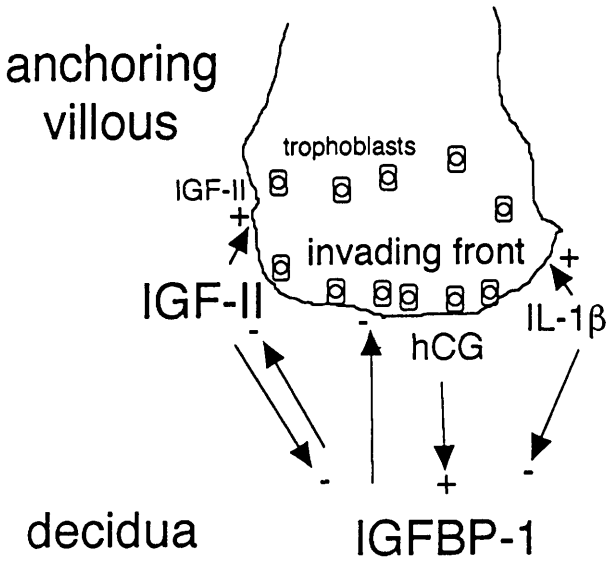

Figure 4. The human placental bed, illustrating the potential roles of IGF-II, hCG, IL-1β, and IGFBP-1 in the regulation of trophoblast invasion into the maternal decidua (see text for details).

diated *via* its internal RGD sequence (*vide supra*). The invading trophoblast, unlike other trophoblast phenotypes, expresses the $\alpha_5\beta_1$ integrin (332) or fibronectin receptor, which is postulated to be the binding site for the IGFBP-1 RGD domain. Recent studies demonstrate that IGFBP-1 binds specifically and inhibits fibronectin binding to this integrin in trophoblasts (15). Such an action could theoretically suppress fibronectin inhibition of trophoblast cell migration, thereby potentiating the invasive process. IGFBP-1 has also been shown to stimulate migration of passaged human trophoblasts on a plastic surface (17). However, trophoblast invasion into decidualized endometrial stromal cell multilayers actually appears to be suppressed in the presence of IGFBP-1 (15). A possible explanation for this latter finding might be found in the observations that IGF-I and IGF-II are potent stimulants of cell migration, and IGFBP-1 inhibits IGF-binding to cell surface receptors. These opposing *in vitro* observations probably reflect differences in experimental conditions and cell populations. However, within the endometrium *in vivo,* IGFBP-1 inhibition of IGF-stimulated cell migration may predominate over IGFBP-1 blockade of fibronectin action, resulting in net suppression of trophoblast invasion. In this way, IGFBP-1 may act as a maternal "restraint" on trophoblast invasion.

An interesting recent finding is a selective inhibitory effect on IGFBP-1 and prolactin secretion in decidualized endometrial stromal cells plated on laminin (333); other secretory products were apparently unaffected. Laminin is a major component of the endometrium during decidualization and pregnancy and is thought to be essential for normal cell adhesion. Although the mechanisms for the laminin effects on IGFBP-1 and prolactin are undefined, laminin actions in other systems may be mediated by laminin binding to integrins.

A possible role for IGFBP-1 during pregnancy is also

suggested by the strikingly high levels found in maternal and fetal fluids (334). IGFBP-1 levels in pregnant maternal serum are elevated 2-fold or more relative to nonpregnant serum; however, these levels are still several orders of magnitude lower than in amniotic fluid (4, 335). Fetal serum levels are intermediate between maternal serum and amniotic fluid concentrations. The patterns of maternal and amniotic fluid IGFBP-1 levels are roughly parallel, increasing to a peak at midgestation, decreasing slightly thereafter, and increasing again near term (336–339). Limited data indicate that fetal serum levels follow a similar pattern (4). Early gestation (6–13 weeks) maternal serum IGFBP-1 levels have been correlated with maternal serum hCG and human placental lactogen concentrations, and negatively with maternal weight (340). The IGFBP-1 present in these fluids is intact and capable of binding ligand; fragments of IGFBP-1 have not been identified. Maternal serum IGFBP-1 levels are higher in twin as compared with singleton pregnancies, are similar between twin and multifetal pregnancies, and decrease toward singleton-pregnancy levels following embryo reduction, suggesting that maximal IGFBP-1 levels are achieved with twin pregnancy (341).

IGFBP-1 concentrations and phosphorylation patterns have been studied to gain insight regarding the origin of this protein during pregnancy. The origin of IGFBP-1 in amniotic fluid is probably the maternal decidua, as indicated by several lines of evidence. Phosphorylation patterns of amniotic fluid and decidual IGFBP-1 parallel one another from early through late gestation (231). In the first trimester, the extra-embryonic coelom (EEC), lined by chorion/decidua, has extremely high levels of IGFBP-1, and first trimester amniotic fluid has relatively low levels (335, 342). However, upon transitioning into the second trimester, the amnion and chorion/decidua fuse, effectively obliterating the EEC. It is at this time that concentrations of IGFBP-1 increase several orders of magnitude in amniotic fluid, probably transported from the decidua across the chorion and amnion.

It is not clear whether phosphorylation plays an *in vivo* role in the regulation of IGFBP-1 action; however, distribution of the phosphoisoforms may be useful markers of sites of production. In maternal serum, IGFBP-1 exists in a highly phosphorylated state and in partially and nonphosphorylated isoforms (28, 343). Since IGFBP-1 in nonpregnant serum circulates as a single, highly phosphorylated species primarily of hepatic origin (28), it has been suggested that this form of IGFBP-1 in pregnancy serum is also of hepatic origin and that the partially and nonphosphorylated variants, present in lower concentrations, are of decidual origin. This latter contention is supported by the failure to detect highly phosphorylated forms of IGFBP-1 in amniotic fluid and the presence of the partially and nonphosphorylated variants in amniotic fluid and decidua (28, 29, 343). Parallel, gestational age-related changes in IGFBP-1 phosphoisoform patterns in serum, amniotic fluid, and decidual explants further implicate the decidua as the major contributor to amniotic fluid IGFBP-1 and a source of IGFBP-1 in pregnant maternal serum (28, 29, 343).

Pre-eclampsia is a common, serious disorder of pregnancy, occurring in 5%–10% of pregnant women and characterized by maternal hypertension, proteinuria, and increased vascular permeability (344–346). If left undiagnosed or untreated, pre-eclampsia can result in maternal multiorgan failure, coagulopathy, seizures, and both fetal and maternal mortality. In the industrialized world, pre-eclampsia is a major cause of fetal and maternal morbidity and mortality. A consequence of this disorder is a generalized placental hypoxia, and cytotrophoblast invasion into the uterine decidua is abnormally shallow (347, 348). This may result from abnormal trophoblast adhesion molecules (348) and/or elevated decidual levels of IGFBP-1 preventing deeper placental invasion (349). In support of the latter hypothesis is the clinical finding that, in pregnancies complicated by severe pre-eclampsia, maternal serum IGFBP-1 levels in the second and early third trimesters are ~6-fold higher (349) and at term are ~2-fold higher than in normal pregnancies (350–352). Although nonspecific elevation of hepatic proteins often occurs in pre-eclampsia, maternal serum levels of IGFBP-3, also largely of hepatic origin, are normal or low and IGFBP-3 is not a useful predictor of pre-eclampsia (352, 353). Moreover, a significant correlation was observed among maternal diastolic blood pressure, aspartate transcarbamylase, and IGFBP-1, suggesting that IGFBP-1 reflects severity of pre-eclampsia and hepatic involvement.

It appears likely that the elevated maternal serum IGFBP-1 in severe pre-eclampsia derives from both the decidua and liver. In support of this are preliminary data that in pregnant women with hepatic disease, the highly phosphorylated form of IGFBP-1 is markedly elevated (Giudice and Martina, unpublished data). In the decidua, IGFBP-1 is found in the periarteriolar region; enhanced vascular permeability and vasospasm in pre-eclampsia would account for increased release of IGFBP-1 from the decidua into the maternal circulation.

In a recent longitudinal study (354), maternal serum IGFBP-1 levels were decreased at midgestation in women who subsequently developed mild pre-eclampsia. However, it is likely that mild and severe pre-eclampsia are different clinical disorders with different pathogenetic mechanisms. It is still controversial as to whether maternal serum IGFBP-1 levels in early gestation may predict development of severe pre-eclampsia in mid or late gestation.

Relatively low maternal serum IGFBP-1 levels and lack of the normal midgestational peak has been demonstrated in women with ovum-donation pregnancies (355). The authors postulated that this may be due to lack of normal endogenous ovarian function and resultant deficiencies in estrogen, progesterone, and relaxin. Early and late gestation maternal serum IGFBP-1 concentrations were normal as compared with natural pregnancy, as were fetal outcomes,

implying that the apparently lower midgestational IGFBP-1 production did not have a detrimental effect on pregnancy.

Premature ovulation or elevated levels of luteinizing hormone (LH) in the follicular phase may lead to premature endometrial decidualization. Moreover, elevated concentrations of LH have been associated with increased risk for spontaneous abortion (356). Whether elevated production of IGFBP-1 by the decidua predisposes to poor implantation and miscarriage has yet to be determined. However, measurements of IGFBP-1 in maternal serum do not appear to be related to preterm delivery (339).

Fetal growth. Fetal growth is a complex process influenced by genetic, nutritional, environmental, placental, and maternal factors, each of which can have profound effects on fetal outcome (357). Fetal weights below the 10th percentile for gestational age are associated with greatly increased risks for severe morbidity and mortality (358). Both the fetal and maternal IGF systems are involved in fetal growth (42, 359, 360). In IGF-I and IGF-II gene knockout studies, mouse pups are born with marked growth retardation (361, 362). Human studies have demonstrated a direct correlation between cord blood or fetal serum IGF-I concentration and fetal size or birth weight (363, 364). Moreover, although contradictory data have been published (365), several investigations have noted a striking inverse correlation between fetal size and maternal serum or fetal blood IGFBP-1 concentrations (339, 352, 363, 366–374). In a study of 200 pregnancies, maternal serum IGFBP-1 levels at both 20–24 weeks and 30–34 weeks gestation correlate inversely with fetal size as assessed by ultrasound at each sampling period (375); fetal size was also correlated with maternal serum glucose concentrations during oral glucose tolerance testing. Cordocentesis at 26–27 weeks demonstrated >2-fold elevated IGFBP-1 concentrations and 4-fold lower IGF-I levels in fetuses with uteroplacental insufficiency ($n = 140$) as opposed to small ($n = 22$) or appropriate ($n = 94$) for gestational age fetuses (376). Similar, but much less dramatic, changes were observed in the corollary maternal serum samples. A study of cord blood from 19- to 35-week-gestation fetuses showed an inverse correlation between IGFBP-1 and IGF-I concentrations (377). In pregnancies resulting from superovulation (pituitary desensitization with buserelin coupled with human menopausal gonadotropin induced superovulation) followed by *in vitro* fertilization and embryo transfer, third trimester maternal serum IGFBP-1 levels were elevated, and birth weights were lower as compared with natural pregnancies (378). Increased first trimester maternal serum IGFBP-1 has been noted in pregnancies complicated by fetal trisomy 18, a condition associated with decreased early-gestational fetal growth, but not in trisomy 21, which is not associated with early gestational fetal growth disorder (379). Overall, these data indicate a role for IGFs in fetal growth and for IGFBP-1 as an inhibitor of this growth.

In rats, IGFBP-1 is expressed at relatively high levels in hepatocytes during the perinatal period. Rat fetal IGFBP-1 mRNA is detectable at high levels in early gestation, appearing shortly after albumin expression, increases during gestation, remains relatively elevated until birth, then declines abruptly ~3 weeks after birth (4, 380–384). A similar pattern of developmental change has been described for rhesus monkeys, where hepatic IGFBP-1 mRNA levels are high in fetal and neonatal samples and become nearly undetectable in liver from pubertal and adult monkeys (385). Developmental changes in IGFBP-1 transcription may be due to developmental changes in the expression of nuclear proteins which interact with the IGFBP-1 promoter (386). These and other observations (387, 388) indicate that the primary source of circulating IGFBP-1 in the fetal circulation is likely the fetal liver and production is regulated at the level of hepatic mRNA abundance. Small amounts of IGFBP-1 mRNA have also been detected in human fetal kidney (204, 388); however, this is unlikely to contribute significantly to the circulating pool. IGFBP-1 has not been detected in other fetal tissues (389).

The stimulus for elevated IGFBP-1 in the maternal or fetal circulations in pregnancies complicated by intrauterine growth retardation has not been determined, although it has been suggested that elevated glucocorticoid levels associated with fetal distress may be contributory in the setting of growth restriction due to placental insufficiency (390, 391). While this is an attractive hypothesis, *in vivo* and *in vitro* data in nonfetal models indicate that glucocorticoid stimulation of IGFBP-1 production is potently inhibited by physiologic levels of insulin which, in turn, is postulated to be an important growth factor during fetal life (230, 357). Therefore, a more probable theory to explain the elevated fetal serum IGFBP-1 concentrations in intrauterine growth retardation might involve abnormally low fetal insulin levels. In relation to this hypothesis, IGFBP-1 levels are increased in fetal sheep during maternal starvation, and return to normal following insulin or glucose treatment (369, 392, 393). Infusion of catecholamines into fetal sheep led to a marked increase in fetal liver IGFBP-1 expression (394), which might argue for a pathogenetic role for stress-related catecholamine release. However, the catecholamine infusions also resulted in significantly decreased serum insulin concentrations. Similarly, hypoxia-induced increases in fetal IGFBP-1 mRNA and protein levels could be mediated by reduced insulin concentrations (395). Furthermore, isolated rat fetal islet cells have been shown to respond to nutrient availability and IGFs, indicating early ontogenesis of control mechanisms for insulin release (230). An inverse relationship between cord blood IGFBP-1 and insulin was reportedly found in small and appropriate for gestational age human fetuses at 26–27 weeks but not in fetuses with uteroplacental insufficiency, although the complete data were not presented (376).

A possible exception to the relationships among fetal growth, IGFBP-1, and insulin was observed in severely malnourished or untreated streptozotocin-induced diabetic

pregnant rats, in which both IGFBP-1 and insulin concentrations were depressed and fetal growth was inhibited (396); these observations may be due to the limited absolute level of substrate availability. Muaku *et al.* (397) reported that maternal protein-calorie restriction resulted in fetal growth retardation and reduced fetal IGF-I mRNA and serum levels, whereas protein restriction alone had no effect. IGFBP-1 levels were reported to be unaffected; however, IGFBPs were analyzed by ligand blot and IGFBP-1 and IGFBP-2 bands could not be distinguished. Perhaps related to these models, Gallaher *et al.* (398) reported that periconceptual maternal undernutrition could lead to a subsequent blunting of the fetal IGFBP-1 response to malnutrition; the mechanism for this effect has not been determined.

The prediction of fetuses at risk for development of intrauterine growth retardation would be extremely valuable in designing clinical prevention and treatment protocols. Limited data indicate that IGFBP-1 concentrations in second trimester amniotic fluid are predictive of subsequent low birth weight (399). Prospective trials are needed to confirm the potential use of IGFBP-1 in fetal assessment and surveillance.

Increased cord blood IGFBP-1 levels were observed during labor as compared with samples from infants delivered by caesarean section (e.g., before the onset of labor), and levels were higher in deliveries complicated by meconium staining of the amniotic fluids, while concurrent maternal serum IGFBP-1 levels did not vary with labor conditions (391). Wang *et al.* (339) reported a similar comparison; however, in this study, maternal serum IGFBP-1 levels were higher in the normal labor group as compared with caesarean section, whereas cord blood levels were not different. In addition, maternal IGFBP-1 levels were inversely related to insulin in both groups, but cord blood levels were not. These results indicate that acute stress at delivery can elevate IGFBP-1 levels, and that this may be regulated by insulin. However, given the discrepant results, further studies will be needed to determine whether this effect is primarily at the fetal or maternal level.

Premature rupture of membranes with leakage of amniotic fluid is a major cause of peripartum maternal and fetal morbidity and mortality and prompt detection of this complication is essential for effective treatment. Amniotic fluid contains IGFBP-1 levels which are several orders of magnitude higher than pregnant maternal serum (4). This observation prompted the study of IGFBP-1 determinations in detection of premature rupture of membranes. Using vaginal swab specimens analyzed by IGFBP-1 EIA, Lockwood *et al.* (400) found that elevated IGFBP-1 has a positive predictive value for premature rupture of membranes of 96.7%. In separate studies, IGFBP-1 had a positive predictive value of 95% as compared with 79% for measurements of fetal fibronectin (401). Similar results have recently been reported by Ragosch *et al.* (402) and Woltmann *et al.* (403).

Ovary. The ovary is a site of IGF, IGF receptor, and IGFBP expression and action. IGFs stimulate ovarian cellular mitosis and steroidogenesis and IGFBPs are, in general, inhibitory to these processes (404). Five of the six known IGFBPs (IGFBP-1 through 5, but not IGFBP-6) have been identified in human ovary (323, 405, 406). However, IGFBP-1 has not been found in porcine granulosa cells (407, 408). IGFBP-1 is found in follicular fluid (FF) from gonadotropin-stimulated luteinizing follicles and in FF from normally cycling women (409, 410). It is likely that IGFBPs in FF result from a combination of local ovarian production and transudation from serum. In support of local production are data that luteinizing granulosa cells in culture synthesize and secrete IGFBP-1. IGFBP-1 is expressed in granulosa cells of the dominant follicle following the LH surge (411), and IGFBP-1 mRNA is abundantly expressed in corpora lutea (323, 405). IGFBP-1 inhibits IGF-I-stimulated [^{3}H]thymidine incorporation into DNA as well as progesterone and E_2 production in human granulosa cells. *In vivo,* IGF-II production predominates over IGF-I in the corpus luteum and IGF-II may be the physiologic target for the inhibitory action of IGFBP-1. IGF-II has been reported to be more potent than insulin in inhibiting IGFBP-1 production by luteinized human granulosa cells *in vitro* (156).

Human estrogen dominant FF contains primarily the nonphosphorylated isoform of IGFBP-1 (232), and this is the form that is produced by granulosa-luteal cells *in vitro* (412), indicating that FF IGFBP-1 is produced by these cells *in vivo.* Moreover, IGFBP-1 concentrations were found to be higher in FF as compared with serum in women undergoing treatment for infertility (413). IGFBP-1 production by cultured nonluteinizing and luteinizing granulosa cells may be stimulated by growth hormone (414) and is reported to be inhibited in dose-dependent fashion by insulin and IGFs through the insulin and type I IGF receptor, respectively (156, 415, 416, 417) and FSH (415). In addition, exogenous IGFBP-1 blocks IGF-I binding and inhibits estradiol production in luteinizing human granulosa cells *in vitro* (415). In luteinizing human granulosa cells *in vitro,* follicle-stimulating hormone inhibits IGFBP-1 secretion; this effect may be mediated by FSH-stimulated IGFBP-3 proteolysis which would lead to increased IGF bioactivity (418).

By immunostaining, IGFBP-1 appears to be the most abundant IGFBP in stromal and tubal smooth muscle cells of the fallopian tube, with lower levels during the late secretory and early proliferative phases of the menstrual cycle and in the postmenopausal period (419). The source and function of IGFBP-1 in this structure are unknown.

Circulating IGFBP-1 has been reported not to change during the normal menstrual cycle (420–422) or to have a pre-ovulatory peak and a second peak at the onset of menstrual shedding (423). With exogenous gonadotropin stimulation, circulating IGFBP-1 levels increase as multiple ovarian follicles develop, with the highest serum IGFBP-1 levels occurring at the time of oocyte retrieval (424, 425). During ovulation induction for fertility therapy, luteal phase levels of IGFBP-1 are about 2-fold higher in women receiving clomiphene citrate alone and about 3-fold higher in those

receiving both clomiphene citrate and gonadotropins as compared with spontaneously ovulatory and anovulatory cycles (421). In women receiving human menopausal gonadotropin for fertility therapy, serum IGFBP-1 concentrations were significantly higher in those subjects with an exuberant follicular and/or estradiol response (426). Subsequent treatment of these subjects with follicle-stimulating hormone resulted in significantly lowered IGFBP-1 concentrations. It is uncertain whether the luteinizing follicles, decidualizing endometrium, or liver are primary contributors to these increased levels, and it is yet unknown whether the IGFBP-1 concentrations are predictive of corpus luteum function or pregnancy success.

Polycystic ovarian syndrome (PCOS), the most common cause of anovulation in women in the industrialized world (427), is characterized by hyperandrogenism, persistent anovulation, and oligo- or amenorrhea. It is often accompanied by hirsutism, elevated LH levels, and the accumulation of small follicles in the ovaries (427–429). Both obese and lean women with PCOS are commonly hyperinsulinemic. In the PCOS ovary, the initial stages of follicle development are not impaired; however, selection of a dominant preovulatory follicle does not occur, resulting in accumulation of small antral follicles (429). Ovulation can be induced with antiestrogens in about 70% of women with PCOS; ~30% require gonadotropins for ovulation induction, and resistance to exogenously administered gonadotropins is often observed clinically (429). Despite extensive research, basic mechanisms underlying this disorder remain unclear.

Insulin and the IGF family are believed to be involved in the pathogenesis of PCOS (430–432). Specifically, high levels of insulin are believed to synergize with elevated levels of LH, acting on theca and resulting in increased ovarian androgen production, characteristic of this syndrome. While elevated levels of insulin acting *via* the type I IGF receptor in theca have been proposed as an etiology of hyperandrogenism in PCOS (433), the insulin levels required *in vivo* for this effect would be higher than the levels which are typically observed in PCOS. In addition recent studies with human granulosa cells from normal and polycystic ovaries reveal that insulin action in these cells is mediated by the insulin receptor and not the type I IGF receptor (434) and PCOS granulosa are not insulin resistant (435). Moreover, reduction of plasma insulin concentrations with pirenzipine did not alter plasma testosterone or androstenedione levels in women with PCOS (436).

The insulin, somatotropic, and LH axes in lean and obese women with PCOS are intimately related with a state of relatively low levels of growth hormone, elevated levels of insulin, free IGF-I, and LH, and low levels of IGFBP-1 (194, 437–441). IGFBP profiles in PCOS FF appear to be normal (442, 443). It has been suggested that the decreased levels of circulating IGFBP-1 in PCOS (4, 108, 194, 444), a reflection of hepatic response to insulin, contributes to elevated free IGF-I levels in this syndrome.

Therefore, the IGF system in PCOS is believed to increase the bioavailability of IGF-I to the theca *via* lowered IGFBP-1 concentrations, which in turn are related to increased insulin concentrations (4, 445). IGF-I may then act synergistically with LH to increase ovarian androgen production (446). Treatment of PCOS with oral contraceptives results in decreased circulating levels of LH, increased serum IGFBP-1, and decreased IGF-I, and this is associated with decreased androgen production, while oral contraceptives (combined ethinylestradiol 35 mcg and 2 mg cyproterone acetate) do not cause these changes in women who have normal ovulatory cycles (447, 448).

Laparoscopic ovarian electrocauterization can lead to resumption of normal ovulatory cycles in some women with PCOS, and this is associated with reduced serum LH and androgen concentrations with no change in insulin or IGFBP-1 (449). The mechanism by which electrocauterization restores ovarian function is not defined, but could be related to local destruction of androgen-producing tissue.

IGFBP-1 abnormalities have been reported in other disorders of ovarian function. Adolescent girls with insulin-dependent diabetes mellitus (IDDM) and irregular menses were reported to have lower IGF-I and higher IGFBP-1 levels than girls with IDDM and normal cycles (450). This was associated with higher body mass index, higher hemoglobin A_{1c} levels and polycystic ovarian changes. Furthermore, in a study of elite athletes and dancers with exercise-related amenorrhea or menstrual irregularity, elevated IGFBP-1 was found to account for 67% of the statistical variance in menstrual irregularity, with elevated cortisol levels accounting for most of the residual variance; insulin, IGF-I, and adrenal and ovarian hormones did not contribute significantly (451). Although more detailed confirmatory data is needed, it is interesting to speculate that the increased serum IGFBP-1 levels may inhibit estradiol production and normal ovarian function.

Unlike the situation with uterine endometrium, IGFBP-1 has not yet been implicated in the pathogenesis of ovarian malignancy. IGFBP-1 has not been detected in human ovarian carcinoma cell lines (452) or in primary cultures of human epithelial ovarian carcinoma cells (Conover *et al.*, unpublished data).

Breast. IGFBP-1 mRNA has been identified in estrogen-receptor (ER) negative breast cancer cell lines, but not in ER-positive lines; expression of other IGFBPs in these lines has also been found to be ER-related (212). IGFBP-1 has been shown to inhibit the growth of MCF-7 breast cancer cells in response to exogenous IGF-I, estrogen, and serum, and to endogenously produced IGF-I *via* inhibition of binding to the type I IGF receptor (236, 453). IGFBP-1 expression was not found in R3230AC mammary adenocarcinoma tumors implanted in normal or insulin-deficient rats (454).

IGFBP-1 has not been conclusively identified in rat milk (455). and was not visualized by ligand blot in human colostrum (456). However, IGFBP-1 is detectable by RIA in

human milk and are 10%–20% of concurrent plasma concentrations (153).

Male Reproductive System. Serum IGFBP-1 concentrations are strongly correlated with total and free testosterone, but not with age or sex-hormone binding globulin, in normal young and middle-aged men (457). The mechanisms for these relationships are currently undefined. IGFBP-1 mRNA has not been detected in rat or human testicular tissue (458, 459). Low levels of IGFBP-1 have been detected by immunoassay and ligand blot in seminal plasma, and neither serum nor seminal plasma levels change with vasectomy (460).

IGFBP-1 has not been identified in the human prostate carcinoma cell line, PC3, either before or after stable transfection with androgen receptor (461). In addition, IGFBP-1 had no effect on human sperm motility or binding of human spermatozoa to the zona pellucida, whereas placental protein-14, a product of the decidualized endometrial epithelium (IGFBP-1 is a product of the stroma), inhibited zona binding (462).

Rat PA-III prostate cells, which are known to cause bone metastases when implanted *in vivo,* were found to produce a urokinase which hydrolyses small MW IGFBPs, identified by ligand blot as IGFBP-1 and IGFBP-2, produced by UMR 106 rat osteoblasts *in vitro* (463). The activity of the PA-III protease was inhibited by benzamidine and aprotinin, both serine protease inhibitors. In addition, the PA-III protease enhanced UMR mitogenesis, apparently by hydrolysis of these endogenous small MW IGFBPs leading to reduced inhibition of endogenous IGF-I action; this effect was eliminated by monoclonal antibody blockade of the type I IGF receptor. The authors postulated that production of urokinase by prostate cells could enhance the metastatic process and the local osteoblastic reaction. Immunologic identification of the small MW IGFBPs involved in this process is needed. A similar phenomenon has not yet been reported in human tissues.

Kidney. Although IGFBP-1 mRNA and protein have been identified in monkey and rat kidney (454), identification in postnatal human kidney has been difficult. IGFBP-1 mRNA is found in extremely low levels during human embryonic nephrogenesis (464) and has been localized to the parietal epithelial cells of the glomerular capsule in sections of apparently normal adult human kidney (465). This is in contrast to adult rat, in which IGFBP-1 mRNA localizes to the distal nephron (466).

In a rat model, unilateral nephrectomy resulted in a prompt and sustained 6-fold increase in IGFBP-1 mRNA expression in the remaining kidney (467). This was accompanied by increased expression of IGFBP-2 and the type I IGF receptor. IGFBP-1 is markedly increased in rat distal nephron following furosemide-induced increase in renal work load (468). Renal IGFBP-1 mRNA is also increased in mercuric chloride-induced acute renal failure in IGF-I–treated rats despite decreased levels of serum IGFBP-1 (469). The increased renal IGFBP-1 expression observed in these studies is similar to that seen in liver regeneration (4) (*vide supra*), and suggest that IGFBP-1 may have a role in compensatory kidney hypertrophy and tissue recovery.

Renal IGFBP-1 mRNA is elevated in rats with spontaneous GH deficiency, declines with GH treatment, and increases with fasting (470). These effects are probably mediated by insulin, which was not measured in these studies, and are virtually identical to the regulatory responses of hepatic IGFBP-1 observed in other investigations. Specific insulin regulation of renal IGFBP-1 mRNA and protein expression has also been reported in rats with insulin-deficient diabetes (92) and renal IGFBP-1 mRNA was increased in protein-restricted rats (130). In rats with streptozotocin-induced diabetes mellitus, IGFBP-1 mRNA increased >2-fold in renal cortex and decreased in the usual site of renal IGFBP-1 expression, the medullary thick ascending loop (471). Limited data suggest that IGF-I accumulation in renal membrane of diabetic rats may be due to trapping by IGFBP-1 expressed in the kidney rather than to local IGF-I production (472).

Children and adults with chronic renal failure (CRF) have normal or elevated serum levels of GH, IGF-I, and IGF-II; however, serum IGF bioactivity is low (4, 473, 474). This paradox is primarily due to increased levels of unsaturated serum IGF binding activity and IGF binding proteins (475–480). IGFBP-1 levels, measured by immunoassay, ligand blot, or immunoprecipitation techniques are high in sera from patients with CRF as compared with age-matched controls (152, 474, 476–478, 481, 482); the degree of elevation is often several orders of magnitude above controls and correlates inversely with glomerular filtration rate (474). Furthermore, the elevated levels of IGFBP-1 are apparently due to intact, bioactive protein rather than to immunoreactive fragments (481). Elevated levels of urinary IGFBP-1, as determined by ligand blot, have also been reported in children with CRF (483, 484).

Our previous studies have demonstrated that serum IGFBP-1 levels in CRF are directly correlated with serum IGF-binding activity (481), implying that IGFBP-1 may play a key role in dynamic inhibition of serum IGF bioavailability. In this respect, it is of note that IGFBP-1 has been inversely correlated with measures of serum free IGF-I in CRF children with growth failure (152) and in non-CRF populations (77, 149a, 485, 486). It is interesting to speculate that elevated serum and extravascular IGFBP-1 concentrations might account for the abnormal linear growth associated with pediatric CRF (reviewed in Ref. 477). IGF-I and IGF-II can stimulate linear growth in rats (487) and exogenously administered IGF-I stimulates linear growth in humans with functional GH receptor deficiency (488); both lines of evidence support the hypothesis that IGFs mediate the skeletal growth effects of GH *in vivo.* Since serum IGFBP-1 binds IGFs with affinities higher than those reported for the membrane-bound type I IGF receptor (reviewed in Ref. 489), excess IGFBP-1 *in vivo* may inhibit IGF binding

to the type I IGF receptor and thereby suppress the cell metabolic effects of IGFs.

Several investigations lend support to the hypothesis that excess IGFBP-1 can inhibit linear bone growth. IGFBP-1 is present in peritoneal dialysate, a fluid which approximates extravascular fluid, at ~10% of serum levels, or ~2 n*M* in children with CRF (490). This level is 10-fold higher than the 0.2 n*M* IGFBP-1 concentration required to inhibit basal growth of chick embryo pelvic cartilage in organ culture (234). Moreover, in the hypophysectomized rat model GH or IGF-I stimulated weight gain and tibial epiphyseal widening; these effects are inhibited in a dose-dependent manner by co-administration of recombinant, nonphosphorylated hIGFBP-1 (221). In two separate experiments, rhIGFBP-1 inhibited GH-stimulated tibial epiphyseal widening by >50%.

In a large population of children with CRF, IGFBP-1 showed a statistically significant inverse correlation with height *z* score (474); GH treatment leads to a dramatic acceleration in height velocity accompanied by a ~50% fall in serum IGFBP-1 concentrations (152, 474, 482, 491). The fall in serum IGFBP-1 levels was accompanied by a rise in serum insulin levels, suggesting that insulin mediates the inhibitory effect of rhGH on IGFBP-1 in this population (152). Interestingly, serum IGFBP-2 concentrations had a stronger inverse correlation with height *z* score in CRF children than did IGFBP-1 (152, 474), indicating that IGFBP-1 is probably not the only IGFBP contributing to growth failure in CRF.

In addition to negative effects on skeletal growth, excess levels of IGFBP-1 might be expected to inhibit other IGF-mediated metabolism in both children and adults with CRF. Although it is interesting to speculate that this mechanism may contribute to some of the metabolic abnormalities associated with this condition, supportive data has not yet been published.

The etiology of the abnormally high IGFBP-1 levels in CRF has not been determined. Rats with renal failure, due to 5/6 nephrectomy exhibit growth failure, normal serum IGF-I levels by RIA and 4-fold elevated serum IGFBP-1 levels by RIA (492), results which are similar to findings in children with CRF (*vide supra*). In these rats with CRF, hepatic IGFBP-1 mRNA levels were elevated 2-fold, suggesting that much of the increase in serum IGFBP-1 may be due to increased hepatic IGFBP-1 expression; this possibility has not yet been explored in human CRF. Supranormal levels of IGFBP-1 expression in CRF could result from hepatic insulin resistance; however, inverse relationships of fasting IGFBP-1 and insulin appear to be preserved (152). Acute regulation of IGFBP-1 by insulin in CRF has not yet been reported. Normalization of serum IGFBP-1 levels following successful renal transplantation (493) might argue for decreased clearance of IGFBP-1 and/or thus-far unidentified substances which stimulate hepatic IGFBP-1 expression as a mechanism(s) for the pretransplant elevated IGFBP-1 concentrations.

Bone, Cartilage, and Connective Tissue. IGFBP-1 mRNA was not identified in primary cultures of rat calvarial or parietal cells enriched for osteoblasts (494, 495), rat osteoblastic MC3T3-E1 cells (496), rat bone marrow stromal cells (497), or developing murine bone or cartilage (498). However, normal human osteoblastic (hOB) cells in culture were recently shown to express IGFBP-1 mRNA and protein when exposed to glucocorticoid (91, 499). Insulin, acting through insulin receptors on these cells, inhibited basal and glucocorticoid-stimulated IGFBP-1. IGFs were equipotent inhibitors of IGFBP-1 expression in hOB cells, and may act through type I IGF receptors on these cells.

IGFBP-1 mRNA was not found in human dermis or epidermis from normal human adult chest (500) or in bovine or human skin fibroblasts with or without glucocorticoid exposure (501). IGFBP-1 mRNA has been found in low abundance in early passage human epidermal keratinocytes; however, IGFBP-1 production by these cells has not been demonstrated (502).

IGFBP-1 has been implicated in inhibition of type I (skin) and type II (cartilage) collagen synthesis in vitamin C–deficient and fasted guinea pigs (503, 504). In both models, type I and type II collagen mRNA concentrations decreased rapidly with weight loss, and this decrease was temporally related to decreased insulin levels, increased serum levels of unsaturated IGF-binding activity, and increased IGFBP-1 levels.

IGFBP-1 has been reported to enhance IGF-II–mediated stimulation of wound re-epithelialization in normal human skin organ cultures in the presence of fetal calf serum (505). In this model, neither IGF-II nor IGFBP-1 had independent effects and IGFBP-1 did not affect IGF-I–mediated stimulation of wound healing. Topical IGFBP-1 also appears to enhance IGF-I–mediated healing of experimentally induced wounds *in vivo* (506); a small IGF-independent effect of IGFBP-1 was also noted in this study. In addition, IGFBP-1 enhances the IGF-I–stimulated increase in breaking strength of incisional wounds in a rat model (233). Finally, IGFBP-1 significantly enhances IGF-I–mediated fibroblast contraction *in vitro,* while it does not have IGF-independent action in this system (507). These results could be due to IGFBP-1–mediated prolongation of IGF-I half-life and may be applicable to the design of therapeutic agents for wound healing, but their relevance to IGFBP-1 physiology is uncertain.

Potential extravascular actions of IGFBP-1, such as at wound sites, would depend upon the presence of IGFBP-1 in the extravascular compartment. However, direct demonstration of IGFBP-1 in normal interstitial or wound fluid has not been achieved. As a possible reflection of interstitial fluid, Xu *et al.* (508) sampled fluid from mechanically produced epidermal pressure blisters in normal volunteers. By ligand blot, the band identified as IGFBP-1 was much lower in blister fluid as compared with serum. As discussed in previous sections, IGFBP-1 has been identified in peritoneal

fluid from healthy women (298) and in peritoneal dialysate from children with chronic renal failure (490); both of these fluids are also hypothesized to be similar to interstitial fluid. However, it is not clear whether IGFBP-1 enters these extravascular spaces *via* active and/or regulated mechanisms or by passive transport or leakage; the former option might favor a physiologic role for IGFBP-1 in the extravascular compartment and, in particular, at sites of wound healing.

Obesity and Insulin-Related Disorders. In a previous report (73), we found that hyperinsulinemic obese subjects had low fasting serum IGFBP-1 concentrations which were inversely correlated with insulin, and that the expected increase in IGFBP-1 was blunted during a hypoinsulinemic, euglycemic clamp study. Similar results have been reported by Bang *et al.* (509), who found that IGFBP-1 failed to increase during fasting in hyperinsulinemic patients with non–insulin-dependent diabetes mellitus (NIDDM), whereas increases were noted for normal and nondiabetic obese subjects (baseline fasting serum IGFBP-1 levels were comparable among the three groups in this study). Low integrated IGFBP-1 and GH secretion have also been reported in obese adults (510, 511), and GH treatment of obese women leads to a further decrease in serum IGFBP-1 concentration (512). The inverse relationship between insulin and IGFBP-1 is similar in normal, normal-obese, and both obese and non-obese women with polycystic ovary syndrome (73, 436), indicating that the decreased IGFBP-1 levels seen in hyperinsulinemic obesity is due to the hyperinsulinemia and not obesity *per se.* Decreased IGFBP-1 levels could play a pathogenetic role in obesity by increasing the levels of free IGF-I, which in turn could suppress GH secretion and contribute to the insulin-like activity in serum (4, 512, 513).

In NIDDM, serum IGFBP-1 concentrations are usually decreased in relation to hyperinsulinemia (4). Gibson *et al.* (514) found that different standard therapies for NIDDM can have divergent effects on IGFBP-1. Sulfonylurea treatment was associated with decreased serum IGFBP-1, increased serum pro-insulin levels, and an apparent loss of meal-related IGFBP-1 fluctuations. Patients treated with metformin or daily insulin had IGFBP-1 patterns similar to nondiabetic controls. Multiple-dose insulin therapy was associated with increased IGFBP-1 levels.

Hyperinsulinism and insulin resistance are thought to be major factors in the pathogenesis of cardiovascular disease (515), and detection and treatment of these conditions could have individual and public health benefits. However, these metabolic abnormalities are often asymptomatic even when manifest as NIDDM. Furthermore, serum insulin and glucose concentrations are known to be unreliable in screening for this disorder, particularly in the absence of NIDDM. Recently, Mogul *et al.* (516) showed that reduced serum IGFBP-1 concentrations are an excellent predictor of integrated insulin secretion and are highly predictive of hyperinsulinism in obese women without NIDDM. Reduced serum IGFBP-1 levels have also been correlated with several cardiovascular risk factors in NIDDM subjects (517). Hellénius *et al.* (518) found that serum IGFBP-1 levels increased dramatically during a 6-month intervention with diet, exercise, or diet and exercise in men with increased cardiovascular risk factors. The IGFBP-1 increase was more dramatic than the decreases in pathologic oral glucose tolerance tests, decreases in serum insulin, or increases in serum IGF-I. On the other hand, 3-week treatment of obese subjects with fluoxetine, which increases whole body insulin sensitivity (as measured by the minimal model method) had no effect on serum IGFBP-1 concentrations (519). More extensive, prospective studies will be needed to confirm the utility of IGFBP-1 in clinical screening and in monitoring treatment programs for hyperinsulinism and associated conditions.

Fasting serum IGFBP-1 and low-density lipoprotein (LDL) levels were correlated in a study of 41 healthy adults (520). By multivariate analysis, IGFBP-1, body mass index, and sex were all found to be independent predictors of LDL. However, although insulin was measured and is likely to provide a link among these variables, it was apparently not included in the statistical analyses.

Serum IGFBP-1 concentrations are elevated in IDDM and have been related to poor glycemic control (521–523). Although insulin regulation of IGFBP-1 is clearly preserved in IDDM (4, 105, 524), the absolute relationships between IGFBP-1 and insulin in this disorder remain controversial (122, 148, 521) (*vide supra*). Increased IGFBP-1 and decreased serum insulin levels may be major contributory factors to the morning rise in blood glucose levels (''dawn phenomenon'') in adolescents with IDDM (525).

Glucocorticoid Excess. We have previously shown that, during hypoinsulinemia, glucocorticoids stimulate IGFBP-1 production *in vivo* (107). Fasting serum IGFBP-1 concentrations were comparable to normal controls in patients with Cushing's syndrome (526). Inverse correlations of IGFBP-1 with IGF-I and urinary cortisol secretion were also noted; the latter may be secondary to increased insulin levels although C-peptide of insulin concentrations were not related to IGFBP-1. Four days of dexamethasone treatment in normal subjects resulted in increased insulin concentrations and decreased IGFBP-1; an independent effect of dexamethasone to increase serum IGFBP-1 was not observed (527).

IGFBP-1 were normal in 19 children following renal transplantation and were not different between the daily and alternate-day prednisone (0.15 mg/kg/day) treatment groups (480). Glucocorticoid treatment of children with Crohn's disease led to decreased serum IGFBP-1 concentrations, but growth rate was not improved (528).

Infection, Surgery, and Trauma. Injection of endotoxin (*Escherichia coli* lipopolysaccharide) in rats led to an acute and prolonged increase in plasma IGFBP-1 concentrations which were unrelated to insulin levels, which showed no significant change (529). The mechanism for this paradoxical effect was not defined; however, plasma IGF-I

levels decreased and corticosterone levels increased during the study, both of which could contribute to increased IGFBP-1 levels. Transient elevated levels of serum IGFBP-1 have also been observed in critically ill patients (530).

A paradoxical relationship of IGFBP-1 and insulin has been observed during abdominal surgical procedures, in which both IGFBP-1 and insulin show acute concurrent increases (115), and in chronic liver disease, where both IGFBP-1 and insulin are elevated (244). IGFBP-1 levels also increase in sheep during administration of surgical anesthesia (halothane) as compared with similarly fasted controls; however, insulin levels decreased in both populations (531). Interestingly, anesthesia was also associated with upregulation of liver IGFBP-1 mRNA in pouch young from the Tammar wallaby (*Macropus eugenii*) (532).

As assessed by ligand blot, serum IGFBP-1 concentrations did not change in patients during treatment of severe burn injury (533). However, the identification of IGFBP-1 was inferred from its gel migration characteristics, and was not confirmed by immunological methods. On the other hand, using a specific immunoradiometric assay, Lang *et al.* (534) found ~10-fold increased serum IGFBP-1 levels in burn patients despite nutritional support and elevated insulin concentrations. IGFBP-1 levels decreased in response to insulin infusion, but nadir levels were still 4-fold elevated. However, the changes in IGFBP-1 concentrations were not quantitatively observed using ligand blots or affinity labeling/immunoprecipitation methods, perhaps reflecting quantitative limitations of these latter methods. Sustained elevations in serum IGFBP-1 during nutritional intervention have also been observed in severe trauma patients (535) and in rats receiving total parenteral nutrition (536).

Serum IGFBP-1 concentrations are several-fold elevated in patients with wasting associated with acquired immunodeficiency syndrome (AIDS) and may be associated with decreased levels of IGF-I, insulin, and IGFBP-3 (537, 538). The etiology of wasting in AIDS is controversial, and has been observed to occur even with adequate caloric intake. The possible pathophysiologic role of elevated serum IGFBP-1 concentrations may warrant further investigation.

As discussed in a previous section, the paradoxical relationship of IGFBP-1 and insulin during infection, surgery, and trauma may be related to increased cytokine activity (116). However, detailed *in vivo* studies defining relationships among cytokines, insulin, and IGFBP-1 are needed to further explore this possibility.

Neuromuscular and Nervous System. IGFBP-1 has not been identified in muscle tissue, myometrium, or myocardium (539), in a variety of rat neural cell types *in vitro* (540) or in regenerating rat neurons (541). However, specific IGFBP-1 immunoreactivity has been found at murine neuromuscular junctions and appears to co-localize with markers which bind the acetylcholine receptor (542). IGFBP-1 was also identified in association with neurofilaments and within motor nerve axons. Studies indicate that IGFBP-1 may enter at the synapse and undergo axonal transport (543). The role of IGFBP-1 within peripheral nerves is not known.

No changes in serum IGFBP-1 concentrations were observed in nondiabetic or IDDM patients undergoing stimulation with growth hormone–releasing hormone alone or after administration of cholinergic agents pirenzepine or pyridostigmine, indicating that cholinergic pathways are probably not involved in acute regulation of serum IGFBP-1 (544).

Although IGFBP-1 inhibits vascular smooth muscle cell mitogenesis *in vitro,* no effect on vascular wall morphology was noted with supraphysiologic infusion of IGFBP-1 during carotid artery balloon catheterization injury in rats (215).

IGFBP-1 levels are reported to increase during prolonged exercise. This is accompanied by significant decreases in serum glucose and insulin concentrations in subjects fed a placebo preparation during the exercise, but not with ingestion of glucose polymer (545). The authors postulated that factors other than glucose or insulin might regulate serum IGFBP-1 during exercise. However, an inverse correlation between insulin and IGFBP-1 was observed in the placebo group. The lack of a relationship between IGFBP-1 and insulin during glucose polymer ingestion could have been related to variability in the timing of blood sampling in relation to endogenous changes in glucose, insulin, and IGFBP-1 concentrations. Similarly, although serum IGFBP-1 levels are reported to increase with unchanged insulin concentrations following a marathon run, both levels were measured simultaneously and the dynamic relationships between these analytes may have been missed (546). Exercise is also reported to increase serum IGFBP-1 concentrations in fasted rats (70). However, IGFBP-1 levels did not increase during 8 weeks of endurance training in older men and women (547).

IGFBP-1 mRNA has not been identified in normal brain, but has been found in malignant brain tissues (548, 548a). IGFBP-1 causes a dose-dependent inhibition of mitochondrial reductase activity in ovine fetal brain cells cultured in 5% fetal plasma without added IGF (549, 550). In addition, brain growth was disproportionately retarded in IGFBP-1 transgenic mice (43, 222). These results suggest that IGFBP-1 could play a role in regulating the growth of brain tissue. However, although serum IGFBP-1 levels may be elevated in protein-calorie–deprived neonatal rats during a period of cerebellar growth retardation, IGFBP-1 was not identified in cerebellar tissue (549).

IGFBP-1: *In Vivo* Physiology

As reviewed in this manuscript and in our previous review (4), our understanding of IGFBP-1 physiology is based upon a complex mixture of *in vitro* and *in vivo* data. Careful consideration of the information supports and extends the *in vivo* models of IGFBP-1 action which we discussed in 1993. New elements include possible effects of

IGFBP-1 on cell adhesion and migration, thus far demonstrated only *in vitro,* and the emerging understanding of relationships between cytokines and IGFBP-1. In this section we bring together the available data to speculate on models of IGFBP-1 action which may be useful in future studies. The reader is asked to refer to previous sections and to our previous review for details.

IGFBP-1 and Substrate Metabolism. There is overwhelming evidence that IGFBP-1 plays an integral role in glucoregulation. At the gene level, similarities between the promoter regions for IGFBP-1 and PEPCK and the dominant transcriptional regulation of IGFBP-1 production by insulin demonstrated both *in vitro* and *in vivo* strongly support such a role. Detailed *in vivo* studies by Lewitt and colleagues (219), observations in IGFBP-1 transgenic mice (222, 224, 292), and increasing evidence of an inverse relationship between IGFBP-1 and levels of free IGF-I in serum also add to the model. Peripheral administration of IGF-I increases free IGF-I levels and has substantial effects on lipogenesis and amino acid uptake; these effects may also be regulated by IGFBP-1.

During fasting, insulin levels fall and IGFBP-1 rise precipitously. This rise is undoubtedly due to a decreased insulin-inhibitory effect on IGFBP-1 gene transcription, and may be augmented by increased levels of counter-regulatory hormones such as cortisol and glucagon, and, in some circumstances, cytokines. The net result is increased levels of serum IGFBP-1, decreased levels of free IGF-I and decreased insulin-like actions of IGF-I on peripheral metabolism. On the other hand, in the fed state, insulin levels increase, IGFBP-1 levels fall, free IGF-I levels rise, and insulin-like actions of IGF-I are augmented. The importance of these mechanisms in normal glucoregulation and other elements of substrate utilization are not defined. However, current estimates of the free fraction of serum IGF-I, ~1%–10% of total concentrations, are well within the range that could affect tissue metabolism; estimated levels of free or easily dissociable IGF-I in serum after a typical overnight fast are ~100–1000 p*M,* while fasting levels of insulin are typically <100 p*M.* Insulin peaks are substantially higher after a meal; these fluctuations are episodic and associated with decreased IGFBP-1 and further increases in estimated free or dissociable IGF-I.

A dual effector model involving insulin and free IGF-I (and, possibly, free IGF-II) is evident, in which both circulating hormones regulate basal and postabsorptive metabolism, each targeted to certain cells and tissues according to the distributions of the IGFBPs and the insulin and IGF receptors. Insulin, which is directly regulated by substrate availability, may provide an acute metabolic response mechanism in the postabsorptive state, with actions that include suppression of IGFBP-1 production, which in turn increases the potential metabolic activity of circulating IGFs. On the other hand, during basal or fasting conditions, lack of insulin suppression causes a dramatic increase in serum IGFBP-1 concentration, which leads to a suppression of IGF activity. Other considerations, such as the direct effects of IGFs on insulin and IGFBP-1 production, the stimulatory effect of insulin on overall IGF-I production, and the "critical set-point" characteristics of insulin suppression of IGFBP-1 production (as opposed to a true dose response) (4, 73) add to the complexity of this model. Overall, the picture is one of insulin acutely regulating substrate utilization during the postabsorptive state, both directly and by increasing IGF bioavailability through suppression of IGFBP-1 expression. During the fasting state, low levels of insulin may place a limit on peripheral metabolism, and this may be further augmented by the consequent increase in serum IGFBP-1, leading to decreased levels of free IGF-I. This interplay of IGF, insulin, and IGFBP-1 may have relevance to clinical pathophysiology in the pathogenesis of obesity, which is associated with increased serum insulin, decreased IGFBP-1, and increased free IGF-I (*vide supra*). On the other hand, increased levels of serum IGFBP-1, as well as other IGFBPs, in chronic renal failure may contribute to decreased peripheral substrate utilization, skeletal growth, and tissue metabolism.

Although the cytokine-mediated disruption of the insulin/IGFBP-1 relationship and increased IGFBP-1 levels during trauma and infection may seem to contradict the hypothesis detailed above, this paradox could have positive effects on tissue recovery and repair. Increased levels of serum IGFBP-1 in these situations would lead to decreased free IGF-I in serum, thereby inhibiting generalized IGF-mediated peripheral metabolism. This may then preserve substrate availability for sites of tissue repair, where local levels of free IGF-I may be increased (551–556). Further investigations are needed to explore this hypothesis.

IGFBP-1, Reproduction, and Tissue Growth. As reviewed above, the localized cyclic changes in IGFBP-1 expression which occur in the uterine endometrium and ovary during normal menstrual cycles can be regarded as the early steps of a continuum which, following fertilization, involves production of IGFBP-1 by the decidua and IGFBP-1 mediation of blastocyst implantation. We do not yet have a complete understanding of the role of IGFBP-1 in implantation or maintenance of pregnancy; however, the localization of IGFBP-1 expression and its juxtaposition with expression of IGFs, cytokines, and other regulatory factors strongly suggest that IGFBP-1 is an active participant in this system. This conjecture is supported by the extraordinarily high decidual IGFBP-1 production and the extremely elevated concentrations in amniotic fluid.

Fetal serum IGFBP-1 levels are also relatively elevated compared with the postnatal situation. A physiologic role for IGFBP-1 in the fetal circulation is suggested by the inverse relationships between fetal serum or cord blood IGFBP-1 concentrations and fetal size demonstrated in mid and late gestation. The excess levels of IGFBP-1 in fetal serum undoubtedly suppress IGF action in the fetal peripheral circulation and, in this way, regulate IGF-mediated tissue growth in response to substrate availability, insulin, and

other regulatory elements. This model is similar to that described above for a postnatal role for IGFBP-1 in substrate metabolism. Elevated IGFBP-1 concentrations observed in intrauterine growth restriction and low IGFBP-1 levels in infants of diabetic mothers lend support to this hypothesis.

Although *in vitro* data also suggest that IGFBP-1, either alone or as a regulator of IGF action, may be involved in cell attachment and migration, such a role has not been demonstrated *in vivo*. If this action of IGFBP-1 does occur *in vivo*, it would require some level of regulated local expression or transcapillary transport of IGFBP-1 into target tissues. Given the high IGFBP-1 concentrations in fetal serum, fetal development might be an ideal model to test for this hypothesis. However, although IGFBP-1 can affect tissue and whole-body growth in fetal life, there have not been reports of defects related to cell attachment or migration associated with overexpression of IGFBP-1. On the other hand, increased local expression of IGFBP-1 associated with hepatic regeneration and renal injury (albeit in nonhuman species) and apparent localization and axonal transport of IGFBP-1 by peripheral nerves suggest that the actions of IGFBP-1 may not be limited to its role in regulating IGF-mediated tissue metabolism.

Conclusion. In conclusion, we have come a long way in our understanding of IGFBP-1 physiology. The bulk of published data suggest that IGFBP-1 may function primarily as a type of endocrine hormone, released into circulation from the liver in response to peripheral substrate depletion, leading to inhibition of IGF-mediated substrate utilization. In the female reproductive system, IGFBP-1 functions as a paracrine/autocrine factor which is intimately involved in the sequence of events leading from ovulation to normal fetal outcome. Clinical and therapeutic exploration based on these models may be logical steps in future investigations of IGFBP-1.

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