# MINIREVIEW

# Growth Hormone-Binding Proteins (43550B)

GERHARD BAUMANN[1]

*Center for Endocrinology, Metabolism, and Nutrition, Department of Medicine, Northwestern University Medical School, Chicago, Illinois 60611*

Growth hormone (GH) is a polypeptide hormone synthesized and secreted by the pituitary gland under hypothalamic control by GH-releasing hormone and somatostatin. GH is responsible for postnatal somatic growth, but also exhibits a myriad of metabolic actions, including anabolic, calcitropic, lipolytic, anti-insulin, insulin-like, insulotropic, and hematopoietic activities. The growth-promoting/mitogenic activity of GH is mediated by insulin-like growth factor I (IGF-I) in both a paracrine/autocrine and in an endocrine fashion. The multiple, in part seemingly conflicting activities of GH, the absence of a well-defined target organ, and the lack of a unique biochemical response to GH have rendered the investigation of GH action difficult.

The discovery of circulating growth hormone-binding proteins (GHBP) in the mid-1980s opened new and exciting perspectives on growth hormone action. At the same time, they have added an additional degree of complexity to the GH-IGF axis, whose details are only beginning to be understood.

## Discovery of GHBP

GHBP were independently described and characterized in human and rabbit plasma in 1985/1986 in the laboratories of Baumann in the United States (1, 2) and Herington in Australia (3, 4). Several years earlier, Peeters and Friesen (5) had reported a GH-binding factor in the serum of pregnant mice, but this finding was not further explored until after the existence of human and rabbit GHBP had been established. The possibility that GH in blood may be protein bound was first contemplated in the 1960s (6–8), but since no specific binding component could be identified, it was concluded that any apparent binding was artifactual (9). Prevailing dogma dictated that polypeptide hormones circulated in the free form, and despite occasional evidence to the contrary (5, 10–13), this dogma persisted. The GHBP were unexpectedly discovered in the process of characterizing circulating GH forms (1, 2) and as an extension of the identification of a soluble, receptor-like GH binding protein in tissues of the rabbit (3, 4, 14). Initial skepticism about the existence of GHBP has given way to considerable enthusiasm among basic and clinical scientists alike. This enthusiasm is in large part based on the relationship between the principal GHBP in plasma and the GH receptor. In addition to the receptor-related, high-affinity GHBP, a second GHBP with lower affinity for GH has been described in human plasma (15, 16). Most of the work to date has been performed with the human GHBP.

## Structural and Functional Properties of the GHBP

The high-affinity GHBP corresponds to the extracellular domain of the GH receptor. Such a relationship was suspected from the outset based on the functional properties of the GHBP which resembled those of the receptor. The molecular size of the GHBP, however, was much smaller than that of the receptor. Without detailed information about the structure of the GH receptor at the time, proof of a relationship had to be indirect. The study of Laron dwarfism, a GH-resistant condition known to be due to absence of GH receptors in liver membranes (17), provided a strong clue as to the receptor-like nature of the GHBP. When plasma from such Laron dwarfs was analyzed for GH-binding activity, no high-affinity binding was detected (18, 19). Experiments with antibodies directed against the rabbit GH receptor demonstrated that such antibodies also recognized the high-affinity GHBP in rabbit and human plasma (20, 21). (In contrast, the low-affinity GHBP

[1] To whom requests for reprints should be addressed at Center for Endocrinology, Metabolism, and Nutrition, Department of Medicine, Northwestern University Medical School, 303 East Chicago Avenue, Chicago, IL 60611.

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was neither abnormally low in Laron dwarfism, nor was it recognized by antireceptor antibodies.) The cloning of the GH receptor and partial sequencing of the GHBP (22, 23) established the colinearity of receptor and binding protein, and identified the GHBP as the extracellular portion of the receptor. This also explained the disparity of molecular size between GHBP and GH receptor.

The natural high-affinity GHBP in human plasma is a 238–246-amino acid, single-chain glycoprotein with an approximate mol wt of 61,000, as assessed by gel filtration, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and Western blotting (2, 4, 15, 24–26). Its precise carboxy terminus is not known with certainty. The protein backbone accounts for only about half the molecular weight, with the rest being due to carbohydrate (and possibly other, noncharacterized moieties). It forms a complex of 80–85 kDa with human GH under native conditions, as determined by gel filtration (2, 15, 16, 27). Crosslinking studies have provided slightly smaller molecular weight estimates (15, 25, 26), presumably because of constriction by intramolecular crosslinks (15). From these findings, a binding stoichiometry of 1:1 has been derived. (It should be noted here that a highly pure complex of recombinant GHBP with GH can exhibit 2:1 binding [i.e., two binding proteins to one GH] [28; see below]). A complex of the appropriate size for 2:1 binding has thus far not been described in plasma—a disparity that requires further study. The GHBP is an acidic protein with a pI of 5.0 (15); it binds human GH (22-kDa form) with high affinity ($K_a \sim 3\text{–}9 \times 10^8\ M^{-1}$, depending on the technique used) and limited binding capacity (~0.9 nmol/liter of plasma on average). Both pituitary GH and placental GH are bound with identical affinity (29). At 37°C, the association rate constant is $2 \times 10^7\ \text{min}^{-1}$ and the dissociation rate constant is $0.037\ \text{min}^{-1}$ (2, 30), allowing rapid complex formation *in vivo*.

Expression of a recombinant high-affinity GHBP in *Escherichia coli* has permitted a detailed analysis of its structure, at least in its nonglycosylated form (31). The recombinant binding protein binds GH with the same affinity and specificity as the natural binding protein, which suggest that the carbohydrate moiety is not important for the binding function (31). There are three small disulfide loops clustered near the hormone binding site (31, 32); the seventh cysteine near the carboxy terminus is free and may be involved in cleavage of the GHBP from the receptor (see below). The GHBP is folded into two domains, each composed of two antiparallel $\beta$-sheets of four and three strands, respectively (33). The amino-terminal domain is involved in GH binding, whereas the carboxy-terminal domain is in contact with a second GHBP in the 2:1 binding protein-GH complex (33). The model derived from alanine-scanning mutagenesis of GH and GHBP and from the crystal structure of the complex indicates that one GH molecule binds two binding protein molecules via two distinct binding sites on its surface (32, 33). On the other hand, the same binding interface is used on both binding protein molecules to make contact with GH at the two sites (33). This hormone-induced dimerization of the binding protein/receptor is important for biologic signaling of the cell-associated GH receptor (34), but as indicated above, the existence of a 2:1 complex between native GHBP and GH in plasma still needs to be demonstrated. It should also be mentioned that the full-length receptor has an affinity for GH that is about 10-fold higher than that of the GHBP (22, 23). Thus, there are some unexplained differences between the behavior of natural GHBP in plasma and recombinant (nonglycosylated) GHBP in near-ideal solutions, and between the behavior of full-length receptor and GHBP.

A second GHBP has also been described in human plasma (15). It is also specific for human GH, but has lower affinity ($K_a \sim 10^6\ M^{-1}$) and a high binding capacity. It is a larger (~100 kDa) and more basic protein (pI 7.1) than the high-affinity GHBP (15). One study reported a mol wt of 165,000–174,000 (16). The structure of this GHBP is not known in detail; it appears to be unrelated to the high-affinity GHBP or the receptor (15, 19, 35). This GHBP contains a specific binding site for the 20,000-dalton variant of human GH (20K) (36). (Alternatively, it may be a heterogenous mixture that contains a 20K-specific binding protein.) In contrast, 20K binds poorly to the high affinity GHBP or the GH receptor in human liver (2, 36–38).

GHBP have also been found in the blood of several animal species. In the rabbit, a GHBP homologous to the high-affinity GHBP in humans has been well characterized (20, 22, 23). This binding protein also corresponds to the extracellular domain of the GH receptor, and it is indeed the only GHBP where a substantial portion of the amino acid sequence obtained from purified natural GHBP has been published (22). The rabbit protein has not been subjected to the same degree of structural scrutiny as the human GHBP. Nevertheless, it is probably fair to state that the rabbit and human GHBP have very similar structure. The rabbit GHBP binds human GH with a $K_a$ of $3\text{–}6 \times 10^9\ M^{-1}$ (22, 23). GHBP have also been demonstrated in mouse and rat blood (5, 39–43). Structural details about the natural mouse and rat GHBP are lacking, although they also correspond to the extracellular domain of the GH receptor (with a short carboxy-terminal tail), as deduced from mRNA encoding them (44, 45). Several glycosylation states have been described (43, 46). The high-affinity GHBP in rat serum binds human GH and bovine GH with $K_a$ of $9 \times 10^8\ M^{-1}$ and $2 \times 10^8\ M^{-1}$, respectively (40–42). Binding components other than the high-affinity GHBP can be identified in rat blood

(40, 47). Their nature remains unknown. The pig is another species with a prominent circulating high-affinity GHBP (47–49). Based on its size and function, it may also represent the extracellular receptor domain, although little structural information is available. High-affinity GHBP have also been described in poodle (48), chicken, goose, and equine serum (49). Cow and sheep have very little detectable GHBP in their blood (47, 49). Among primates, new world monkeys and macaques show low levels of high-affinity GHBP, whereas the higher apes approach levels seen in humans (G. Baumann, unpublished data). In all these cases, human GH binds with higher affinity than the homologous GH (47, 49; M. A. Shaw and G. Baumann, unpublished).

### Origin of GHBP

There is a single gene encoding the GH receptor, and no gene for the high-affinity GHBP has been identified. Accordingly, the GHBP arises either from the receptor by proteolytic cleavage (i.e., shedding of the extracellular part of the receptor from cells) or from an alternatively spliced mRNA encoding a shortened version of the GH receptor gene transcript. As so often in nature, both possibilities occur. In humans and rabbits, the GHBP is believed to arise from receptor cleavage, as only one mRNA species has been found. In mice and rats, both a full-length receptor mRNA (4 kb) and a shortened version (1.2 kb) are present. The short mRNA codes for a truncated GH receptor with a short hydrophilic tail instead of the transmembrane and intracellular domains (44, 45), i.e., the GHBP. Thus, the GHBP in murine species is believed to be synthesized separately and secreted as such. It is somewhat longer than the human and rabbit GHBP, including a 17 (rat)- or 26 (mouse)-residue carboxy-terminal extension. The GHBP in rat and mouse blood have been shown to be primarily derived from this mechanism, using immunoassays that recognize the unique carboxy-terminal region (46, 50). Little is known about the mechanism of generation of GHBP in other species.

The tissues of origin for the receptor-derived (i.e., cleaved) GHBP are potentially all GH receptor-bearing tissues, although it is unknown whether cleavage occurs to the same degree in each tissue. Very little is understood about the cleavage process; in one model system (IM-9 lymphoblasts), GHBP could be liberated from the receptor by sulfhydryl-inactivating agents at pharmacologic concentrations (51). It is difficult to extrapolate that model to *in vivo* conditions, but it is tempting to speculate that the free cysteine near the transmembrane domain of the receptor is important for cleavage. Since the liver is the organ with the highest GH receptor concentrations, it is likely that it is the main source of GHBP. This is supported by the finding of low GHBP levels in liver cirrhosis (52–54). Nevertheless, other tissues probably also contribute to generation of GHBP. In humans, GH receptors are demonstrable in a variety of tissues by immunohistochemical techniques, and in the rat and rabbit, GH receptors are widely expressed. In the case of a separate mRNA for GHBP, the tissues of origin can be more directly identified. In the rat and mouse, both a 1.2-kb and a 3.9- to 4.5-kb mRNA are identified in numerous tissues, including liver, kidney, lung, adrenal, muscle (skeletal and cardiac), intestinal tract, adipose tissue, and skin (55–58). Correspondingly, GHBP (and/or GH receptors) have been localized in multiple tissues by immunohistochemistry (59). Although the 1.2-kb (GHBP) and the 3.9- to 4.5-kb (GH receptor) mRNA are co-expressed, they are not necessarily regulated in parallel (56–58). Nothing is presently known about the mechanism and regulation of secretion of GHBP.

The source of the *low-affinity* human GHBP is unknown, but liver has been implicated based on abnormal levels in cirrhosis (52). This GHBP is regulated independently of the high-affinity GHBP (52), and, indeed, levels are normal in Laron dwarfism, where both GH receptor and high-affinity GHBP are genetically absent or disabled (19, 35, 60).

High-affinity GHBP have also been demonstrated in rabbit and human milk (61, 62), as well as in human urine (63).

### Regulation of Plasma Levels of GHBP

In humans, plasma levels of high-affinity GHBP fluctuate little throughout the day (64, 65). There appears to be no correlation between a rise in GH and a change in GHBP levels (64, 65), which suggests that GH binding to receptors is not a trigger for GHBP release. One study, however, reported marked fluctuations of GHBP activity when a charcoal-binding assay was used (66). The reason for that discrepancy is not known. There is no significant sex difference in plasma GHBP levels, although women tend to have slightly higher values (16, 27, 52, 67–71). GHBP concentrations are very low in the fetus and neonate (52, 68, 72), and rise rapidly during infancy and progressively slower during childhood and adolescence to stabilize in the late teens (16, 27, 68, 72–74). There is no discernible effect of puberty on this progression. GHBP levels stay constant throughout adult life, at least as judged from cross-sectional studies (16, 52, 70, 72). There is wide variation among individuals, but a given level stays fairly constant over at least a 1-year span (75). Longitudinal studies in individual subjects are required to address the question of long-term trends or fluctuations in GHBP. Human pregnancy, unlike murine pregnancy, does not alter maternal GHBP levels (29, 52, 67, 69). The question of whether GH itself regulates GHBP in humans is unresolved. In adults, neither GH deficiency nor acromegaly (a condition with chronic

hypersecretion of GH by a pituitary tumor) results in substantially altered GHBP levels (2, 52; G. Baumann, unpublished data). In a small number of GH-deficient children, it has been reported that GHBP levels are low and respond to GH supplementation (76, 77). On the other hand, this has not been a consistent observation in a much larger group of such children (74). This is different from murine species, where GHBP and receptor are clearly regulated by GH (78 and references cited therein). Testosterone injections have been reported to decrease GHBP levels (76) and oral, but not transdermal, estradiol treatment to increase GHBP (79).

The low-affinity GHBP in human plasma is regulated differently. Women have higher levels than men, and pregnancy results in further elevation (52). Neonates also have low levels (52) which rise during childhood, but this age progression is not yet known in detail.

In the rat, GHBP levels are higher in females than in males (40, 42, 46). There is an age-dependent increase in both the rat (80, 81) and pig (82). Murine pregnancy results in an at least 30-fold upregulation of plasma GHBP, together with upregulation of the hepatic GH receptor (5, 39, 50, 55, 78). The murine GHBP in both pregnant and non-pregnant animals is induced by GH (78, 83), as is the GH receptor.

**Biologic Actions of GHBP**

The GHBP form complexes with GH in blood. In humans, approximately half of the circulating GH is bound, 40–50% to the high-affinity binding protein and another 5–8% to the low-affinity binding protein (84, 85, 52). This is consistent with theoretical predictions (86). The rate of association is sufficiently rapid to permit these complexes to form within a few minutes after a GH pulse *in vivo* (30, 85). The percentage of GH bound to the high-affinity GHBP declines above a GH level of 15 ng/ml due to saturation of the binding protein (84). The same is not true for the low-affinity complex, which is not saturable at GH levels occurring *in vivo*. Due to continuous association-dissociation, a dynamic equilibrium between GH and the two GHBP exists in the circulation. Because of these dynamics and the slow clearance of bound GH (see below), the circulating GH-binding protein complexes serve as an intravascular hormone reservoir that has a dampening effect on the GH oscillations caused by pulsatile pituitary secretion. An initial mathematical model descriptive of these events has been formulated (30).

GH is cleared from the circulation by glomerular filtration and degradation in the proximal tubule, and also by receptor-mediated internalization into cells, followed by lysosomal degradation. Both of these processes are inhibited by GHBP. The complexes are too large for glomerular filtration, and the high-affinity GHBP inhibits binding of GH to receptors (see below). As a consequence, the metabolic clearance, as well as degradation, of bound GH is about 10-fold lower than that of free GH (87, 88). The distribution volume of bound GH is about twice the intravascular volume, whereas free GH is distributed throughout the entire extracellular space (88). Since the circulation contains a mixture of bound and free GH, the net effect of the GHBP on GH kinetics is intermediate between the extremes of free and bound. Thus, a half-life of *total* GH of 18 min can be calculated to correspond to half-lives of 7 min for free GH and 27 min for bound GH, respectively, at a representative GHBP concentration of 1 n*M* (J. D. Veldhuis, *et al.*, J Clin Invest, in press). The differences in GHBP levels among individuals may in part explain the variation in GH metabolic clearance rates among normal subjects.

The high-affinity GHBP inhibits binding of GH to tissue receptors by competing with receptors for ligand (89, 90). This effect is quite prominent at physiologic concentrations (90) and raises the question as to how GH acts. It should be remembered, however, that 50% or more of plasma GH is free, which is the receptor-active form. Furthermore, the affinity of the full-length receptor for GH is higher than that of the GHBP (22, 23), thereby permitting transfer of GH from the GHBP to the receptor. Nevertheless, the GHBP likely modulates GH action through this mechanism. In contrast to the high-affinity GHBP, the low-affinity GHBP has no inhibitory effect on receptor binding of GH (90).

*In vivo*, GHBP paradoxically enhances rather than inhibits the growth-promoting action of GH (91). This is a dose-dependent phenomenon, ranging from little effect at low doses (91, 92) to marked enhancement at high doses (91). The apparent discrepancy between the *in vitro* and *in vivo* effect of GHBP can be explained by the prolongation of GH half-life, and hence bioavailability, by the binding protein. This effect appears to be dominant over the opposing effect of competition with receptors for GH. It should be recognized that these results were obtained in a rat model with exogenous human GHBP, and that the magnitude of the enhancement of GH action by physiologic GHBP levels in humans is unknown.

One report has demonstrated localization of GHBP in the nucleus (93), suggesting the possibility of a direct action GH or the GH-binding protein complex on gene transcription. Other evidence has linked GH action to c-*fos* and c-*jun* expression (94). Further work is required to define a possible intranuclear role of GH and GHBP.

**Conditions Attended by Altered Plasma GHBP Levels**

Clinical states with GH resistance have provided insight into the biologic significance of GHBP, particularly the high-affinity binding protein. Several such conditions of genetic or acquired origin are associated with low GHBP levels. Laron dwarfism, already men-

tioned above, is characterized by absent, very low, or dysfunctional GHBP (18, 19, 34, 95), a finding that provided the first evidence of the connection between human GHBP and GH receptor (18, 19). The GH receptor gene in Laron dwarfism is either partially deleted (96) or mutated to code for a truncated receptor (97), for a receptor that is not properly translocated to the cell membrane (35, 98), or mutated to create an abnormal splice site causing an 8-amino acid internal deletion (99). The functional consequence in all cases is disruption of GH binding to both receptor and GHBP, although in the last case, the precise mechanism remains to be shown. Heterozygous carriers frequently have GHBP levels/function that are intermediate between normal and homozygous patients (60, 100, 101). Other, less severe conditions of genetic short stature with GH resistance are pygmy dwarfism in Africa (102) and New Guinea (103). These are also attended by decreased GHBP, but levels are not as low as in Laron dwarfism (73, 102, 103). In African pygmies, the regulation of expression rather than the structure of the GH receptor is believed to be altered (73). Reduced GHBP levels have also been described in miniature pigs, which are considered to be GH resistant (48). Several acquired GH-resistant conditions are also accompanied by decreased GHBP levels. Examples are liver cirrhosis (52–54, 67), insulin-dependent diabetes (104, 105), acute fasting (106, 80), chronic malnutrition (107), critical illness (108), and renal insufficiency (52, 109). Low GHBP levels have been reported in hypothyroidism, another condition with impaired growth and a component of GH resistance (110). Finally, preliminary results show that even in the general population, children with so-called idiopathic short stature tend to have decreased GHBP levels (111).

The aggregate of these observations suggests that the high-affinity GHBP is linked to GH action. The most obvious, although unproven, explanation is that the circulating GHBP reflects tissue GH receptor levels, and that higher receptor levels permit more GH action. Studies in the rat and pig, where tissue receptors and GHBP can be measured in parallel, support the view that GHBP can serve as an index for hepatic GH receptor concentration (80, 82). However, it should also be noted that the GHBP may directly enhance GH action independently of the receptor through its effect on GH clearance (91). Thus, GHBP may be linked to GH action either indirectly or directly, or both.

Conditions with increased GHBP levels are less well known. The only one thus far recognized is obesity, where the high-affinity GHBP tends to be high (27, 68, 107, 112). It appears that even within the normal range of body weight, there is a correlation between body mass index (degree of adiposity) and GHBP level (113, 114). It has long been known that obese children grow faster than lean children; this despite the fact that GH secretion is decreased in obesity. The increased GHBP/receptor levels may provide an explanation for this heretofore puzzling observation.

**Physiologic Role of GHBP**

An integrated view of the respective roles of GH secretion and GHBP/receptor in human somatic growth has emerged from the conjoint assessment of both components in normal and GH-deficient children (75, 113). In normal children, an inverse correlation between GHBP and GH secretion rate was found unexpectedly (113). This could be caused either by GH downregulating GHBP, or by GHBP inhibiting GH secretion. To address the question of which of the two components (GH secretion and GHBP/receptor) is the active part in regulating the other, GH-deficient children were studied, where "GH secretion" could be controlled through exogenous GH administration. Interestingly, GHBP levels did not change in response to GH treatment, but the pretreatment level of GHBP was highly correlated with the growth response to a fixed dose of GH (75). Thus, GHBP, and by inference the GH receptor, was suggested as the active component in regulating the above relationship. The presumed factor involved in this negative feedback of GHBP/receptor on GH secretion is IGF-I. These findings further corroborate that the GHBP (and receptor) is a key determinant of somatic growth, and that this determinant is relatively independent of regulation by GH. Based on these observations, the following hypothesis was formulated: In a given person, the GHBP/receptor complement is relatively fixed (perhaps genetically). GH secretion is regulated in a fashion inverse to the GHBP/receptor, presumably through negative feedback by IGF-I, to yield a specific combination of GH secretion and GHBP/receptor level for each person. It is through such a homeostatic mechanism that the genetic growth potential would be assured (75). Nutritional factors, in part through their impact on GHBP/receptor levels (see above), exert an epigenetic effect on this system (114).

It is likely that GHBP have physiologic roles beyond binding of GH in the circulation. Local effects on GH action in tissues are almost certain to occur, although there is presently only scarce information about this. Activities other than GH binding in general should also be considered. This is especially the case for the murine GHBP, which are not directly receptor derived, may be independently regulated (56–58), and bind the homologous GH relatively weakly (39, 40, 42). It is possible that those species that produce GHBP by receptor cleavage and those which synthesize it *de novo* use it for different, if overlapping, purposes.

Little is presently known about the physiologic role of the low-affinity GHBP.

## GHBP and Measurement of GH in Plasma

The presence of GHBP in plasma raises questions about their interference in GH assays. Immunoassays (radioimmunoassays or immunoradiometric assays) are not significantly affected by GHBP because of the much higher affinity of anti-GH antibodies compared with binding proteins (115). In contrast, radioreceptor assays are quite vulnerable to interference by binding proteins because the high-affinity GHBP effectively competes with the receptor for ligand (90). This problem has long been recognized, but its nature not understood, as the "serum effect" in radioreceptor assays. GH results obtained by radioreceptor assay with unextracted plasma should, therefore, be viewed with caution.

## Conclusions

The recognition of circulating GHBP has added a new element of complexity to the GH-IGF axis. At the same time, it has facilitated investigation of that axis. The nature of the high-affinity GHBP as a "circulating receptor" is particularly intriguing. This property has been useful for probing the GH receptor in humans, in whom direct receptor measurements are difficult. The high-affinity GHBP is positively linked, directly or indirectly, to the growth-promoting action of GH. Little is known about the significance of the low-affinity GHBP. The ultimate physiologic role of GHBP still remains to be defined. The development of simple and reliable assays for GHBP (46, 50, 116) that can be applied on a routine basis should facilitate the rapid accumulation of new knowledge in this field.

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