Dynamic Changes of Growth Hormone–Binding Protein Concentrations in Normal Men and Patients With Acromegaly: Effects of Short-Term Fasting

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Plasma concentrations of growth hormone (GH) and GH-binding protein (GHBP) were measured at hourly intervals in five healthy men and five patients with acromegaly during the fed state and after a 5-day fast. GHBP concentrations (both total and complexed with endogenous GH) were analyzed by the ligand-mediated immunofunctional assay (LIFA). Total GHBP was similar in both groups during the fed state (104.4 ± 5.2 and 101.6 ± 10.3 pmol/L), did not exhibit a diurnal rhythm, and was unchanged by fasting (91.9 ± 5.4 and 109.9 ± 10.5 pmol/L, respectively). However, the GHBP/GH complex concentration was significantly higher in acromegalics than in controls (41.0 ± 2.8 *v* 18.0 ± 2.2 pmol/L, respectively; $P < .05$), closely followed diurnal GH rhythm in normals, and was significantly correlated with mean 24-hour GH concentrations ($r = .86$, $P < .01$). We conclude that plasma concentrations of GHBP are stable throughout the day and are unchanged either by short-term calorie deprivation or by chronic exposure to high levels of endogenous GH. In contrast, GHBP/GH complex concentrations are altered both acutely and chronically by ambient GH.

A SPECIFIC BINDING PROTEIN for circulating growth hormone (GH), GH-binding protein (GHBP), has been recently identified in human serum.[1,2] This protein appears to be identical in its amino acid composition to the extracellular domain of liver-membrane GH receptor in several species.[3] Importantly, in vivo serum GHBP levels correlate with in vitro estimates of GH binding to its own hepatic receptor[4,5] and are absent or very low in patients with GH receptor deficiency.[6] Thus, in vivo estimates of serum GHBP may provide potentially important information on the status of the GH receptor that would otherwise require invasive and dangerous interventions, such as liver biopsy.

Regulation of GHBP has been studied in a variety of physiologic and pathologic states in humans,[7-20] but the results are often conflicting. It has been shown, for example, that GHBP levels are low,[11,14] normal,[2,9] or high[21] in patients with GH deficiency and that they increase[11,14] or do not change[9] with GH therapy. Similarly, GHBP was either low[21,22] or normal[4,7] in patients with acromegaly and correlated either negatively[15] or positively[10] with the magnitude of endogenous GH secretion. One possible reason underlying discrepancies in these studies may be related to GHBP assay methodology. Traditionally, GHBP level was estimated indirectly by incubation of a known amount of serum or plasma with radiolabeled GH followed by chromatography or charcoal separation.[23] This methodology may be subject to significant interference by endogenous GH, the contribution of GHBP bound to GH (GHBP/GH complex) cannot be ascertained, and the results are expressed relative to a particular reference serum pool which makes comparison between different assays difficult.

Recently, the ligand-mediated immunofunctional assay (LIFA) specific for GHBP has been developed.[24] This assay uses human recombinant GHBP as a standard and a monoclonal anti–GH receptor immunoglobulin, and allows for accurate and specific quantification of both total GHBP and the GHBP/GH complex. Importantly, LIFA performance is not influenced by endogenous GH. We have used this assay to study GHBP levels in normal individuals and subjects with chronically elevated GH levels (acromegaly). Additionally, we examined the effects of a short-term (5-day) fast on serum GHBP concentrations.

SUBJECTS AND METHODS

Subjects

Five patients with active and previously untreated acromegaly (two men and three women aged 27 to 54 years) and five healthy men (aged 26 to 41 years) participated in the study after signing an informed-consent document approved by the Human Studies Committee of the University of Michigan. Screening complete blood count, liver and kidney function, and calcium, phosphate, and glucose levels were normal in all. All three women with acromegaly had amenorrhea (postmenopausal in two, hyperprolactinemia-related in one). None took any medications except for one woman with acromegaly who was taking L-thyroxine 150 μg/d for the past 20 years, and thyroid hormone concentrations were normal in all. All men had normal plasma testosterone concentrations. All patients and control subjects participated in a previous study that addressed regulation of pulsatile GH secretion by fasting.[25] In brief, they were hospitalized in the Clinical Research Center at the University of Michigan Medical Center for a total of 9 days. During the first 2 days subjects ate a standard hospital diet, and for the next 6 days a total fast was maintained with water provided ad libitum. Assessment of plasma GH and GHBP was conducted on the second fed day and on day 5 of fasting. Plasma GH levels in the original study were measured every 10 minutes for 24 hours (8 AM to 8 AM), and results were reported previously,[25] but for the purposes of the present study only hourly samples were used.

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Assays

Plasma GH level was measured in duplicate by a double-antibody radioimmunoassay, and results have been reported previously.[25] Assay sensitivity was 0.4 μg/L, and concentrations below this level were assigned a numerical value of 0.4 μg/L for purposes of statistical analysis. Plasma insulin-like growth factor-I level was measured by Smith-Kline Laboratories (Van Nuys, CA), and the data were also previously reported.[25]

Plasma concentrations of GHBP (both total and complexed with endogenous GH) were measured in duplicate in hourly plasma samples by the previously described LIFA.[24] All samples for each patient were run in a single assay. Assay sensitivity was 15.6 pmol/L, and concentrations below this level were assigned a numerical value of 15.6 pmol/L for purposes of statistical analysis.

Statistical Analysis

Statistical analysis was performed using a paired or unpaired Student's *t*-test or ANOVA with repeated measures followed by Fisher's test or regression analysis, as appropriate. The level of acceptance for statistical significance was set at P less than .05. Data are shown as the mean ± SE.

RESULTS

A 5-day fast resulted in significant weight loss in both groups (75.8 ± 3.8 to 71.7 ± 3.1 kg in controls and 78.6 ± 4.5 to 73.2 ± 3.2 in patients with acromegaly, $P < .01$ for both). Similarly, plasma insulin-like growth factor-I declined from 221 ± 18 to 111 ± 16 μg/L in normals and from 1,287 ± 82 to 772 ± 54 in patients ($P < .01$ for both).

Normal Controls

During normal feeding, the mean 24-hour plasma GH concentration was 1.03 ± 0.15 μg/L. Many values were near or below the detectability limit, but a clear nocturnal increase in plasma GH to a maximum of 3.8 ± 1.2 μg/L ($P < .05$) was observed between midnight and 3 AM. Mean 24-hour total GHBP was 104.0 ± 5.2 pmol/L without any discernible diurnal pattern. GHBP/GH complex concentrations were less than assay detectability (15.6 pmol/L) most of the time, but increased to a maximum of 35.4 ± 7.4 pmol/L between 1 and 4 AM ($P < .05$). After a 5-day fast,

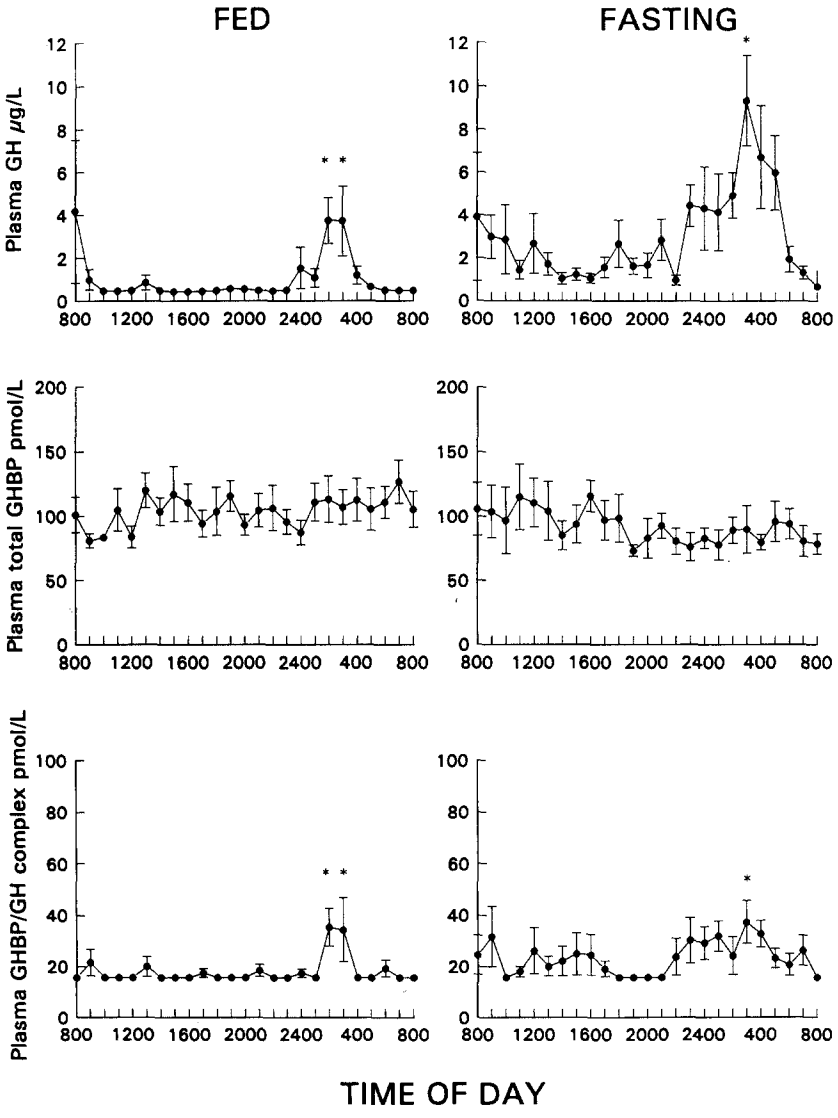

Fig 1. Profiles of plasma GH, total GHBP, and GHBP/GH complex in 5 healthy men. Data are the mean ± SE of hourly samples. *$P < .05$.

mean 24-hour GH concentrations increased to 3.0 ± 0.25 μg/L ($P < .01$ *v* fed state), with all values becoming clearly detectable. The nocturnal increase in plasma GH concentrations was augmented to a maximum of 9.4 ± 2.1 μg/L at 3 AM ($P < .05$). Total GHBP concentrations were not different from those in the fed state (91.9 ± 5.4 pmol/L). In contrast, GHBP/GH complex concentrations increased significantly ($P < .05$) to a mean of 27.3 ± 7.0 pmol/L. The highest GHBP/GH complex concentrations (38.0 ± 9.2 pmol/L) were observed between 11 PM and 4 AM (Fig 1). Examples of GH, GHBP, and GHBP/GH complex profiles in two normal individuals are shown in Fig 2.

Patients With Acromegaly

During the fed period, mean 24-hour plasma GH was 21.3 ± 7.3 μg/L ($P < .001$ *v* controls), and there was no discernible diurnal pattern. Total GHBP (101.6 ± 10.3 pmol/L) was similar to that in normal controls. In contrast, the GHBP/GH complex (41.0 ± 6.2 pmol/L) was significantly higher ($P < .05$) than in controls, with almost all values being above assay detectability. After a 5-day fast, mean plasma GH concentrations did not change as compared with the fed state (19.8 ± 6.9 μg/L). However, whereas in three patients mean GH levels did not change appreciably, they increased from 18.2 to 42.4 μg/L in one patient and decreased from 41.5 to 14.6 μg/L in another. Similar to the normal controls, total GHBP concentrations (109.9 ± 10.5 pmol/L) did not differ from the fed state, and there were no appreciable changes even in the two patients whose mean GH level was markedly influenced by fasting (119.8 to 126.3 and 117.2 to 107.3 pmol/L, respectively). Mean GHBP/GH complex concentrations during fasting did not change as a group (44.2 ± 7.1 pmol/L), and the GHBP/GH complex remained stable in the three subjects whose mean GH level was unmodified by fasting. However, GHBP/GH complex dynamics closely paralleled marked changes in GH concentrations in the other two patients: they increased from 45.5 to 80.7 pmol/L in a patient whose GH level was increased by fasting and decreased from 62.7 to 38.7 pmol/L in a patient whose GH level declined during fasting (Fig 3).

Overall, there was no correlation between mean GH and mean total GHBP concentrations ($r = -.086$). However, there was a strong positive correlation between mean GH level and mean GHBP/GH complex concentration in acromegalic patients ($r = .92, P < .01$) and in the group as a whole ($r = .86, P < .01$; Fig 4). There was no correlation ($r = .27$) between these parameters in normal controls, likely as a result of uniformly low and often undetectable GH and GHBP/GH complex concentrations.

DISCUSSION

Since serum levels of GHBP presumably reflect the abundance of tissue GH receptors,[4] assessment of GHBP in different physiologic and pathologic conditions has been recently undertaken by several groups. Most studies relied on a single-point measurement of serum GHBP level and used an indirect methodology whereby radiolabeled GH was incubated with a known amount of the patient's serum and the bound fraction was separated either chromatog-

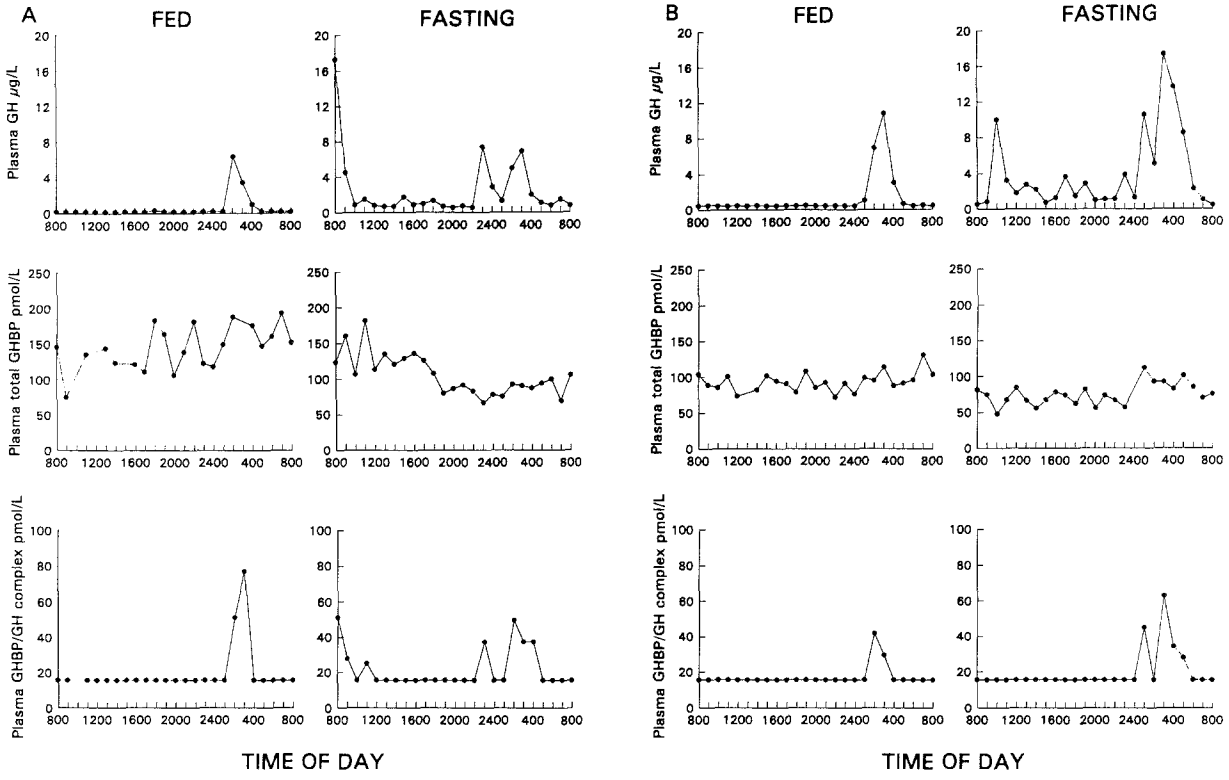

Fig 2. Profiles of plasma GH, GHBP, and GHBP/GH complex in 2 healthy men.

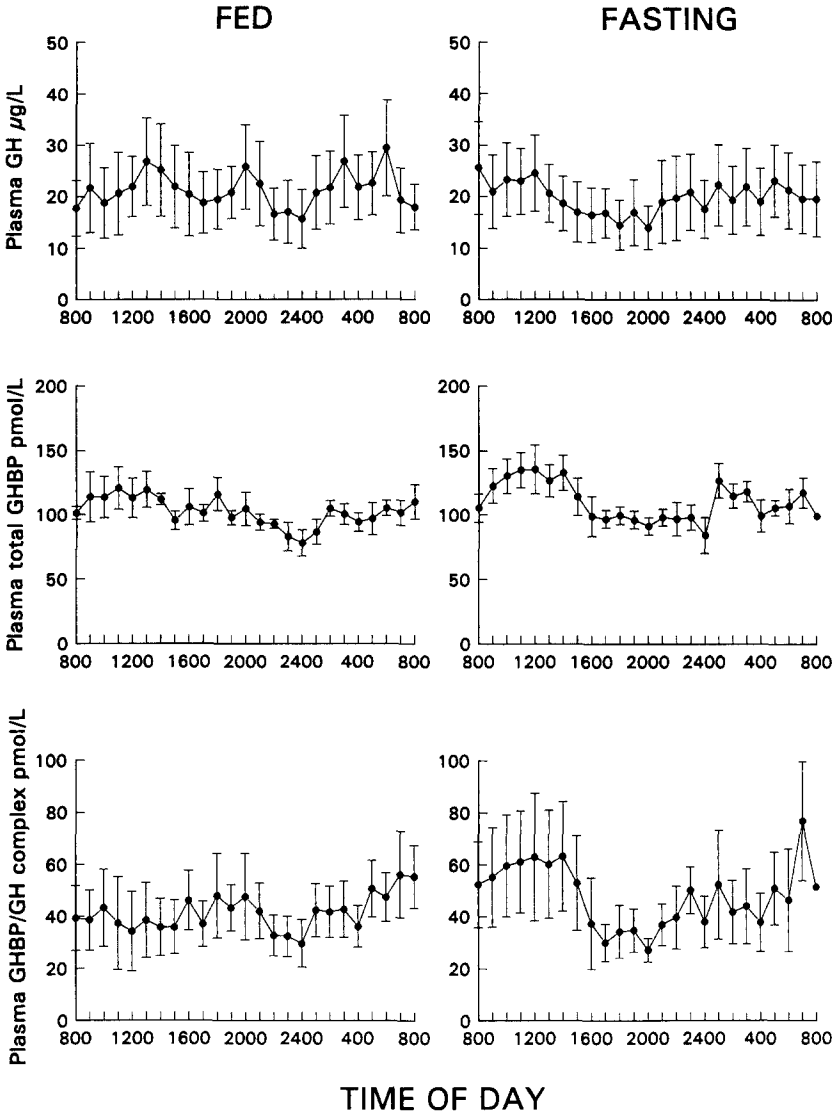

Fig 3. Profiles of plasma GH, GHBP, and GHBP/GH complex in 5 patients with acromegaly. Data are the mean ± SE.

raphically or by the dextran-charcoal method. Probably as a consequence of these shortcomings, previous data on GHBP regulation were conflicting and often contradictory. We used a newly developed LIFA that allows direct measurement of both total and complexed GHBP levels[24] to assess GHBP regulation by short-term fasting and by a chronically high-GH milieu. Additionally, measurement of GHBP levels at hourly intervals throughout the day permitted us to obtain information on the possible diurnal rhythm of GHBP, as well as to ascertain more accurately the prevailing mean GHBP levels.

Similar to previous studies reported by Snow et al,[26] we did not find any evidence for the diurnal rhythm of total GHBP. However, there was a clear increase in the proportion of the GHBP/GH complex coincident with the nocturnal GH increase in normals during both fed and fasting states. Since in our study GH and GHBP levels were measured at hourly intervals, meaningful statistical analysis of GH and potentially GHBP pulsatility was not possible. However, the absence of any total GHBP changes during the nocturnal GH peak makes the possibility of GHBP pulsatility highly unlikely. This is further confirmed by the recent study reported by Carlsson et al,[27] in which GH pulsatility in normal children was accompanied by parallel changes in the GHBP/GH complex and by stable total GHBP levels. Hochberg et al[8] have suggested that serum GHBP fluctuates in a pulsatile fashion, following the endogenous GH pulses. However, it should be noted that in their study, actual increases in GHBP levels were seen approximately 30 minutes after the peak GH level had been measured, similar to their own animal data whereby GHBP pulses lagged behind GH pulses by 60 minutes.[28,29] In view of our data and those reported by Carlsson et al[27] that are based on direct measurement of GHBP and GHBP/GH complex levels, this interpretation is unlikely. Conceivably, the difference may be explained by the possibility that the dextran-charcoal–separation system used by Hochberg et al[28,29] actually measured only the free (unoccupied) GHBP

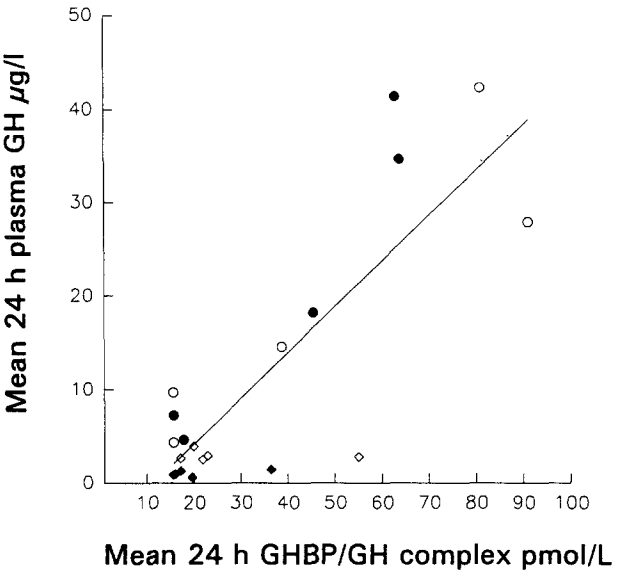

Fig 4. Correlation between mean 24-hour concentrations of plasma GH and GHBP/GH complex in the entire study group. $r = .86$, $P < .01$. Controls: (◆) fed, (◇) fasted. Patients: (●) fed, (○) fasted.

level. If this is the case, an acute increase in the GHBP/GH complex concentration (coincident with a GH pulse) on the background of unchanged total GHBP would result in a temporarily low free-GHBP level. During the decline of plasma GH to normal, reverse changes will occur. Thus, the lag period between GHBP and GH increases observed by Hochberg et al[28,29] may be a methodologic artifact whereby unoccupied GHBP declines rapidly during a GH pulse and then returns to normal, thus mimicking a delayed "pulse."

The possibility of GHBP regulation by endogenous GH has been studied extensively in such models as puberty,[7,15] aging,[10] GH deficiency,[9,11,14] administration of exogenous GH,[9,11,14,16,18] and acromegaly.[2,7,21,22] These studies reached conflicting and sometimes diametrically opposite results. Our data demonstrate that total GHBP is unchanged in acromegaly. This is similar to previous data reported by other groups using LIFA or gel-permeation assays.[2,7,30,31] We did not observe a decrease in GHBP in patients with acromegaly, as was suggested by Hochberg et al[22] and Hizuka et al.[21] However, GHBP/GH complex concentrations were grossly increased in patients with acromegaly and were tightly correlated with the magnitude of endogenous GH hypersecretion. Again, this underscores the point that a significant fraction of GHBP may be occupied by endogenous GH when ambient hormone levels are elevated. This may complicate the performance of indirect assays based on separation of bound hormone from free hormone.

Short-term fasting did not influence total GHBP concentrations either in normal controls or in acromegalic patients. Previous studies in patients with anorexia have demonstrated low total serum GHBP concentrations.[17,19] Counts et al[19] have used the same LIFA as in our study, but their study group included subjects with long-standing malnutrition. Thus, although short-term calorie deprivation does not alter total GHBP concentrations, they may decline with prolonged undernutrition. The exact time course of this phenomenon is uncertain and will have to be ascertained in the future.

In summary, we have demonstrated that total GHBP in humans is not influenced by short-term fasting or prolonged GH hypersecretion and does not exhibit a diurnal rhythm. On the other hand, GHBP/GH complex concentrations may fluctuate markedly both acutely and chronically in parallel with endogenous GH.

REFERENCES

1. Baumann G, Stolar MW, Amburn K, et al: A specific growth hormone–binding protein in human plasma: Initial characterisation. J Clin Endocrinol Metab 62:134-141, 1986

2. Herington AC, Ymer S, Stevenson J: Identification and characterization of specific binding proteins for growth hormone in normal human sera. J Clin Invest 77:1817-1823, 1986

3. Leung DW, Spenser SA, Cachianes G, et al: Growth hormone receptor and serum binding protein: Purification, cloning and expression. Nature 330:537-543, 1987

4. Herington AC, Ymer SI, Tiong TS: Does the serum binding protein for growth hormone have a functional role? Acta Endocrinol (Copenh) 124:14-20, 1991

5. Wallis M: Growth hormone–binding proteins. Clin Endocrinol (Oxf) 35:291-293, 1991

6. Fielder PJ, Guevara-Aguirre J, Rosenbloom AL, et al: Expression of serum insulin-like growth factors, insulin-like growth factor proteins, and the growth hormone–binding protein in heterozygote relatives of Ecuadorian growth hormone receptor deficient patients. J Clin Endocrinol Metab 74:743-750, 1992

7. Baumann G, Shaw MAS, Amburn K: Regulation of plasma growth hormone–binding proteins in health and disease. Metabolism 38:683-689, 1989

8. Hochberg Z, Amit T, Zadik Z: Twenty-four–hour profile of plasma growth hormone–binding protein. J Clin Endocrinol Metab 72:236-239, 1991

9. Daughaday WH, Trivedi B: Clinical aspects of GH binding proteins. Acta Endocrinol (Copenh) 124:27-32, 1991

10. Hattori N, Kurahachi H, Ikekubo K, et al: Effects of sex and age on serum GH binding protein levels in normal adults. Clin Endocrinol (Oxf) 35:295-297, 1991

11. Hochberg Z, Barkey RJ, Even L, et al: The effect of human growth hormone therapy on GH binding protein in GH-deficient children. Acta Endocrinol (Copenh) 125:23-27, 1991

12. Fontoura M, Hocquette JF, Clot JP, et al: Regulation of the growth hormone binding proteins in human plasma. Acta Endocrinol (Copenh) 124:10-13, 1991

13. Baruch Y, Amit T, Hertz P, et al: Decreased serum growth hormone–binding protein in patients with liver cirrhosis. J Clin Endocrinol Metab 73:777-780, 1991

14. Postel-Vinay MC, Tar A, Hocquette JF, et al: Human plasma growth hormone (GH)-binding proteins are regulated by GH and testosterone. J Clin Endocrinol Metab 73:197-202, 1991

15. Martha PM, Rogol AD, Blizzard RM, et al: Growth hormone–binding protein activity is inversely related to 24-hour growth hormone release in normal boys. J Clin Endocrinol Metab 73:175-181, 1991

16. Blumenfeld Z, Barkey RJ, Youdim MBH, et al: Growth hormone (GH)-binding protein regulation by estrogen, progesterone, and gonadotropins in human: The effect of ovulation induction with menopausal gonadotropins, GH, and gestation. J Clin Endocrinol Metab 75:1242-1249, 1992

17. Hochberg Z, Hertz P, Colin V, et al: The distal axis of growth hormone (GH) in nutritional disorders: GH-binding protein, insulin-like growth factor-I (IGF-I) and IGF-I receptors in obesity and anorexia nervosa. Metabolism 41:106-112, 1992

18. Massa G, Bouillon R, Vanderschueren-Lodeweyckx M: Serum growth hormone (GH)-binding protein and insulin-like growth factor-I levels in Turner's syndrome before and during treatment with recombinant human GH and ethinyl estradiol. J Clin Endocrinol Metab 75:1298-1302, 1992

19. Counts DR, Gwirtsman H, Carlsson LMS, et al: The effect of anorexia nervosa and refeeding on growth hormone–binding protein, the insulin-like growth factors (IGFs), and the IGF-binding proteins. J Clin Endocrinol Metab 75:762-767, 1992

20. Menon RK, Arslanian S, May B, et al: Diminished growth hormone–binding protein in children with insulin-dependent diabetes mellitus. J Clin Endocrinol Metab 74:934-938, 1992

21. Hizuka N, Takano K, Asakawa K, et al: Measurement of serum growth hormone (GH) binding protein using anti–GH receptor antibody. Proc Annu Meet Endocr Soc 76:206A, 1990 (abstr)

22. Hochberg Z, Amit T, Bick T, et al: Interrelationship of growth hormone (GH), GH receptor, and GH-binding protein in human and rat. Proc Annu Meet Endocr Soc 249:897A, 1990 (abstr)

23. Baumann G: Growth hormone–binding proteins: Biochemical characterization and assays. Acta Endocrinol (Copenh) 124:21-26, 1991

24. Carlsson LMS, Rowland AAM, Clark RG, et al: Ligand-mediated immunofunctional assay for quantitation of growth hormone–binding protein in human blood. J Clin Endocrinol Metab 73:1216-1223, 1991

25. Ho PJ, DeMott Friberg R, Barkan AL: Regulation of pulsatile growth hormone secretion by fasting in normal subjects and patients with acromegaly. J Clin Endocrinol Metab 75:812-819, 1992

26. Snow KJ, Show MA, Winer LM, et al: Diurnal pattern of plasma growth hormone–binding protein in man. J Clin Endocrinol Metab 70:417-420, 1990

27. Carlsson LMS, Rosberg S, Vitangcol RV, et al: Analysis of 24-hour plasma profiles of growth hormone (GH)-binding protein, GH/GH-binding protein complex, and GH in healthy children. J Clin Endocrinol Metab 77:356-361, 1993

28. Bick T, Amit T, Barkey RJ, et al: The interrelationship of GH, liver membrane GH receptor, serum GH-binding protein activity and IGF-I in the male rat. Endocrinology 126:1914-1920, 1990

29. Hochberg Z, Bick T, Amit T, et al: Regulation by growth hormone (GH) of the GH-receptor turnover. Acta Paediatr Scand [Suppl] 367:148-152, 1990

30. Ho KKY, Valiontis E, Waters MJ, et al: Regulation of growth hormone binding protein in man: Comparison of gel chromatography and immunoprecipitation methods. J Clin Endocrinol Metab 76:302-308, 1993

31. Mercado M, Carlsson L, Vitangcol R, et al: Growth hormone–binding protein determination in plasma: A comparison of immunofunctional and growth hormone–binding assays. J Clin Endocrinol Metab 76:1291-1294, 1993