High-Affinity Growth Hormone (GH)-Binding Protein (GHBP), Body Fat Mass, and Insulin-Like Growth Factor–Binding Protein-3 Predict the GHBP Response to GH Therapy in Adult GH Deficiency Syndrome

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The study objective was to investigate which baseline factors can accurately predict plasma high-affinity growth hormone (GH)-binding protein (GHBP) levels after GH replacement therapy in patients with GH deficiency (GHD). The study group consisted of 36 GHD patients (22 men and 14 women; mean age, 43.1 years; (range, 21 to 60) known to have adult-onset GHD for many years (range, 4 to 22). They were randomly divided into a GH-treated group (n = 19) and a placebo group (n = 17). Body composition (assessed by bioelectrical impendance analysis [BIA]), plasma GHBP (fast protein liquid chromatography [FPLC] size-exclusion gel chromatography), insulin-like growth factor-I (IGF-I), and IGF-binding protein-3 ([IGFBP-3] radioimmunoassays) were measured before and after 6 months. A stepwise multiple linear regression analysis with the plasma GHBP level after 6 months as the dependent variable was used to unravel significant explanatory (or predictor) variables. In contrast to placebo therapy, GH replacement therapy increased the mean plasma levels of IGF-I and IGFBP-3 to the normal range, whereas a small but statistically significant increase in plasma GHBP was observed. The combination of baseline plasma GHBP, body fat mass, and IGFBP-3 predicts posttreatment GHBP levels accurately (adjusted R^2 = .97), indicating that baseline variables such as age, gender, fat-free mass, and IGF-I have no contribution. Furthermore, reliability analysis showed that the observed and predicted values for GHBP fit a strict parallel model. These findings indicate that the variations in body fat mass and IGFBP-3 among adult GHD subjects explain the reported variable response of GHBP to GH replacement therapy.

CIRCULATING GROWTH HORMONE (GH) is partially bound to binding proteins.[1] The high-affinity GH-binding protein (GHBP) is the predominant carrier, represents the extracellular domain of the GH receptor,[2,3] and modulates GH bioavailability.[4] In previous studies, we found that plasma GHBP levels were relatively low in patients with active acromegaly[5] and relatively high in adults with GH deficiency (GHD),[6] suggesting an influence of GH on GHBP. Reports on the effect of GH replacement therapy on plasma GHBP levels in GHD subjects are not consistent: an increase in GHBP levels has been observed,[7-9] as well as unchanged plasma GHBP levels.[10-16] The factors responsible for this varying response of GHBP have not been defined. Recently, we reported that in GHD adults, the GHBP response to GH therapy may be partially determined by the amount of abdominal fat.[17] Fisker et al[18] measured GHBP levels in a group of normal adults in whom data on body composition were also obtained. They concluded that GHBP levels seemed to be determined by abdominal fat mass rather than indices of GH status.

It is well known that adult GHD syndrome is a heterogenous disorder with regard to baseline variables such as age, gender, body fat mass and fat-free mass, and plasma levels of GHBP, insulin-like growth factor-I (IGF-I), and IGF-binding protein-3 (IGFBP-3). We hypothesize that these factors may play a role in the response of GHBP to GH replacement therapy. In recent years, it has been shown that GH replacement therapy in the short- and the long-term improves general well-being and physical activity, decreases body fat mass, and increases lean body mass and bone mineral density.[19] In a 6-month, double-blind, placebo-controlled study on the effects of GH therapy in adult GHD patients, we analyzed the interrelationship between the above-mentioned variables and the plasma GHBP response, to investigate which baseline factors can accurately predict the plasma high-affinity GHBP response to GH therapy. Knowledge of these factors may be of clinical value for proper adjustment of the GH dosage during GH replacement therapy.

SUBJECTS AND METHODS

Patients

Thirty-six adult GHD patients (22 men and 14 women; mean age, 43.1 years; range, 21 to 60) known to have GHD for many years (range, 4 to 22) were included in the study. The aims and methods of the study were explained to all patients, and written informed consent was obtained. The study was approved by the ethical committee of the University Hospital Utrecht. GHD was defined as a peak plasma GH concentration of 5 μg/L or less during arginine infusion (30 g arginine in 30 minutes). Pituitary deficiencies (Table 1) were being treated adequately, and the therapy was not changed during the study. Secondary adrenal insufficiency was present in 33 patients, and was treated with cortisone acetate in a dose of 10.0 to 37.5 mg/d (mean, 29 ± 11 [mean ± SD]). Insufficiency of the pituitary-thyroid axis was observed in 31 patients, and was treated with levothyroxine in a dose of 75 to 200 μg/d (mean, 143 ± 41) to obtain plasma thyroxine levels in the upper range of normal. Eighteen men received testosterone replacement therapy, either testosterone esters 250 mg intramuscularly once every 3 weeks or testosterone undecanoate 80 mg orally twice daily. Eight women with hypogonadotropic hypogonadism received cyclic sex steroid replacement therapy: 30 μg ethinylestradiol and 150 μg levonorgestrel.

Study Design

The patients were randomly divided into two groups according to a random-digits table. One group (n = 19) received GH replacement (Genotropin; Pharmacia, Woerden, The Netherlands) for 6 months. GH was injected subcutaneously once daily, starting with a dose of 0.041

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Table 1. Initial Diagnosis and Pituitary Deficiency in the Patients

Parameter	Men	Women	Total
Initial diagnosis			
Pituitary tumor	16	11	27
Craniopharyngioma	3	1	4
Idiopathic	1	0	1
Head trauma	1	0	1
Sheehan's syndrome	0	1	1
Primary empty sella	1	1	2
Pituitary deficiency			
Isolated GH	1	1	2
GH + ACTH	3	0	3
GH + ACTH + TSH	0	5	5
GH + TSH + FSH/LH	1	0	1
GH + ACTH + TSH + FSH/LH	17	8	25

Abbreviations: ACTH, corticotropin; TSH, thyrotropin; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

mg/kg/wk in the first month and 0.082 mg/kg/wk thereafter. The other group (n = 17) received daily placebo injections for 6 months, which were visually indiscernible from the GH injections. We measured body composition and plasma GHBP, IGF-I, and IGFBP-3 levels after an overnight fast before treatment and after 6 months. Relevant baseline characteristics of both groups are presented in Table 2.

Body Composition

Body weight was measured to the nearest 0.1 kg and height to the nearest 0.01 m. The body mass index (BMI) was calculated as weight divided by height squared. Fat-free mass and body fat were assessed by bioelectrical impedance analysis (BIA) using a tetrapolar BIA-101 analyzer (RJL Systems, Detroit, MI). Resistance and reactance were measured during application of an alternating electric current of 800 μA at 50 kHz with electrodes placed in the middle of the dorsal surface of the left hand and foot.

Biochemical Assays

Serum GHBP levels were measured by fast protein liquid chromatography (FPLC) size-exclusion gel chromatography as previously described.[20] The key feature of this method is that the concentration of GHBP is derived from a six-point Scatchard analysis of binding data. The maximal binding capacity, extrapolated from the Scatchard plot, was regarded as a measure of the GHBP concentration. To exclude the possibility that these binding data were affected by the endogenous level of GH, we tested the influence of increasing concentrations of GH (range, 0 to 80 μg/L) on the GHBP level in pooled plasma, and found that relatively high GH concentrations did not interfere with the measurements of GHBP. At GH levels of 10, 40, and 80 μg/L, GHBP levels were 860, 898, and 854 pmol/L, respectively. Therefore, the measurement of GHBP using FPLC size-exclusion gel chromatography is not significantly affected by the actual level of GH in the serum sample. The GHBP assay had an intraassay coefficient of variation (CV) of 3%, evaluated by multiple (n = 15) incubation of a single sample, and an interassay CV of 11% at a level of 1,000 pmol GHBP/L, evaluated by testing a single sample in 17 different determinations with a full Scatchard plot. Serum IGF-I and IGFBP-3 levels were measured by radioimmunoassay as previously reported.[6] At a concentration of 200 ng IGF-I/mL, the interassay CV was 5.9% and the intraassay CV 7.9%. The intraassay CV for IGFBP-3 was 5.3% and the interassay CV 6.7%. Reference values were 2.1 to 5.2 mg/L, and the lower detection limit was 0.9 mg/L.

Table 2. Baseline Characteristics (mean ± SD) of the GH Replacement Group and Placebo Group

Characteristic	GH Replacement (n = 19)	Placebo (n = 17)	*P*
Male/female (n)	13/6	9/8	
Age (yr)	40.7 ± 12.0	45.4 ± 11.1	.86
Height (m)	1.70 ± 0.09	1.72 ± 0.10	.39
Body weight (kg)	76.0 ± 14.0	75.6 ± 13.5	.82
BMI (kg/m^2)	26.2 ± 4.3	25.4 ± 3.3	.44
Fat mass (kg)	16.1 ± 9.8	20.1 ± 4.9	.50
GHBP (pmol/L)	1,261 ± 350	1,304 ± 262	.83
IGF-I (μg/L)	98 ± 54	70 ± 32	.57
IGFBP-3 (mg/L)	2.2 ± 0.9	1.9 ± 0.9	.42

NOTE. There were no significant differences between the groups (Student's *t* test for unpaired samples).

Statistics

Before analysis, data were transformed to their natural logarithm in the case of skewed distribution. To evaluate differences in baseline data between the GH replacement group and the placebo group, Student's *t* test for unpaired samples was used. Differences in plasma GHBP, IGF-I, and IGFBP-3 levels before and after 6 months were analyzed by Student's *t* test for paired samples, whereas time effects in both groups were compared using a repeated-measures design (SPSS for Windows Release 6.1, ANOVA, Repeated Measures; SPSS, Chicago, IL). Statistical significance was established for *P* values less than .05 (two-tailed). In both groups, Pearson's correlation coefficients between relevant variables such as age, gender, fat mass, fat-free mass, and plasma levels of GHBP, IGF-I, and IGFBP-3 were calculated, followed by a stepwise multiple linear regression analysis (SPSS for Windows Release 6.1, Curve Estimation and Linear Regression) using the plasma GHBP level after 6 months as the dependent variable. The final regression model was tested with a reliability analysis (SPSS for Windows Release 6.1, Scale). All results are expressed as the mean ± SD.

RESULTS

Notwithstanding the random distribution of the patients into two groups, there were no significant differences in baseline data (Table 2). Furthermore, there were no gender-related differences between the groups, and both the baseline BMI and body fat mass were not significantly related to baseline GHBP ($P = .52$ and $P = .76$, respectively). After correction for these covariates (ie, covariates before main effects), we found no main effects of group and/or gender ($P = .36$ and $P = .09$, respectively), nor a significant interaction effect ($P = .09$).

In the GH replacement group, plasma IGF-I and IGFBP-3 levels increased significantly after 6 months, from a baseline of 98 ± 54 ng/mL and 2.2 ± 0.9 mg/L to 280 ± 137 ng/mL ($P < .01$) and 3.7 ± 1.0 mg/L ($P < .01$), respectively. IGF-I and IGFBP-3 levels in the placebo group did not change, from a baseline of 70 ± 32 ng/mL and 1.9 ± 0.9 mg/L to a 6-month level of 69 ± 37 ng/mL ($P = .30$) and 2.2 ± 0.9 mg/L ($P = .55$), respectively. After GH replacement therapy, plasma GHBP increased significantly from 1,261 ± 350 to 1,382 ± 359 pmol/L ($P = .001$). The change in GHBP was outside the range of interassay variation for six of 19 GH-treated patients (Fig 1A). No significant changes in GHBP were found in the placebo group: 1,304 ± 262 pmol/L before and 1,280 ± 281 pmol/L ($P = .39$) after 6 months (Fig 1B). In addition, the placebo group did not show a time effect ($P = .39$), whereas a significant time effect ($P = .001$) was observed in the GH-treated group, demonstrating that a highly significant increase in GHBP

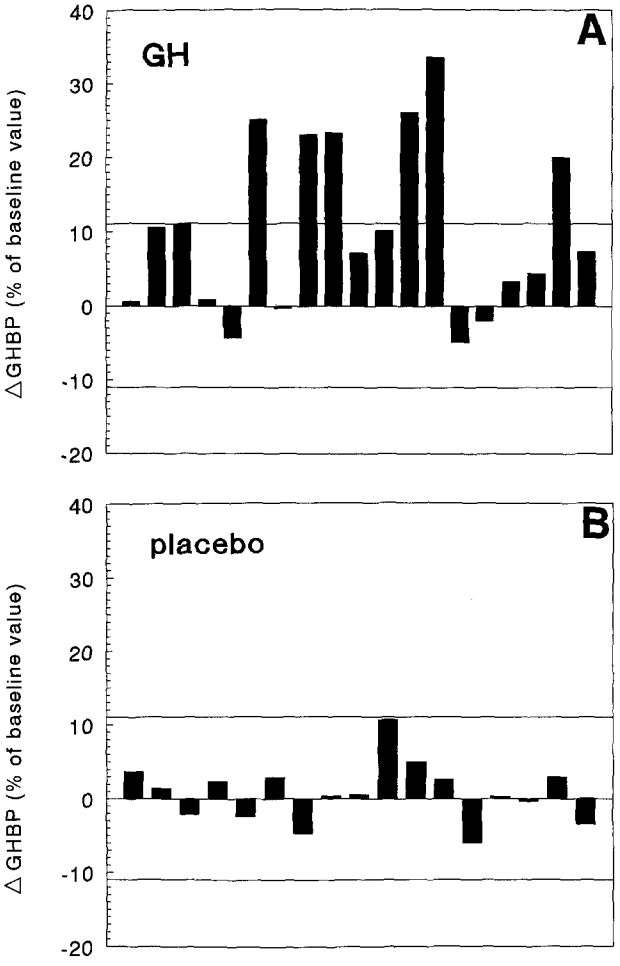

Fig 1. Individual response of plasma GHBP to (A) GH replacement therapy (n = 19) and (B) placebo therapy (n = 17) expressed as a percentage of baseline values. The area between horizontal lines represents the ± interassay CV, which is 11% at 1,000 pmol GHBP/L.

occurred in the GH-treated group only (power at .05 level = .99). Therefore, despite the fact that the effects of GH therapy on plasma GHBP were small, the two groups showed a statistically significantly different response. In the placebo group, a mean decrease in GHBP of 24 pmol/L was observed after 6 months, which is 1.8% of the initial value of 1,304 pmol/L. In contrast, the GH-treated group showed a significant mean increase of 120 pmol/L, which is 9.5% of the initial value of 1,261 pmol/L. The difference in response between the groups (11.3%) is highly significant (multivariate ANOVA with repeated measures, $P < .001$).

Stepwise multiple regression analysis showed that in the GH-treated group, the dependent variable (GHBP after 6 months) can be predicted with an adjusted R^2 of .97 from three baseline variables, ie, GHBP, body fat (ln-transformed), and IGFBP-3, without an additional effect of other variables such as age, gender, fat-free mass, or IGF-I (Fig 2 and Table 3). Moreover, reliability analysis showed that the observed and predicted values for GHBP fit a strict parallel model (chi-square goodness-of-fit, $P = .69$) with an unbiased estimate of reliability of 0.995. A strict parallel model implies that both true and error variances are equal in the observed and predicted values. In a multiple linear regression with the change in GHBP as a dependent variable, we found that body fat mass and IGFBP-3 were independently significantly related to the change in GHBP, with almost exactly the same regression equation as obtained when posttreatment GHBP was used as a dependent variable. The regression coefficients (with standard error) for body fat

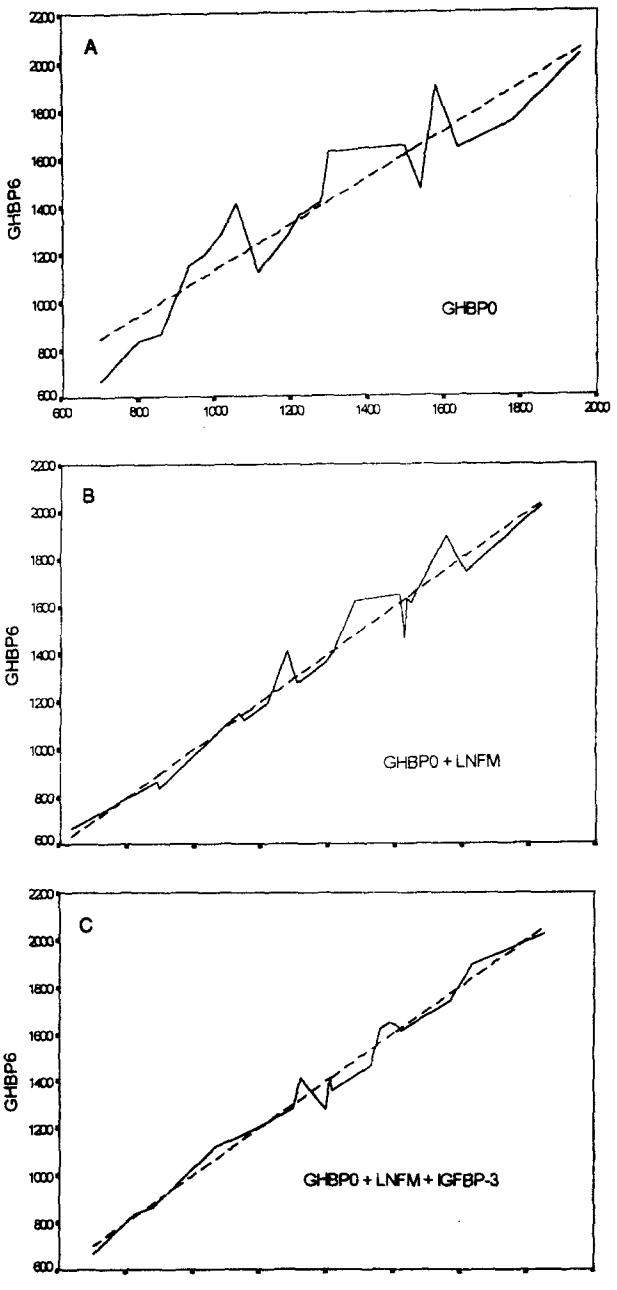

Fig 2. Regression of GHBP after 6 months of GH therapy (GHBP6, pmol/L) on (A) baseline GHBP (GHBP0, pmol/L), (B) GHBP0 and body fat mass (LNFM, kg, ln-transformed), and (C) GHBP0, LNFM, and baseline IGFBP-3 (mg/L). Analysis of curve-fitting showed that a linear model produced the best fit. Differences between observed (—) and predicted (---) data are shown in a group of 19 GH-treated patients. The goodness-of-fit (adjusted R^2) increased from 85% in A to 95% in B, and to 97% in C.

Table 3. Regression Analysis of GHBP After 6 Months of GH Therapy on Baseline GHBP (pmol/L), Body Fat Mass (kg, ln-transformed), and IGFBP-3 (mg/L)

Variable	Coefficient	Standard Error	t	P
GHBP	1.02	0.04	25.85	.001
Ln-transformed body fat mass	177.95	26.43	6.73	.000
IGFBP-3	−61.96	15.30	−4.05	.000
Constant	−242.43	100.03	−2.42	.028

Source of Variation	df	Sum of Squares	Mean Squares	F	P
Regression	3	2,265,626.54	755,208.85	228.50	.000
Residual	15	49,576.09	3,305.07		

mass and IGFBP-3 were 176.23 (25.75) and −60.55 (14.82), respectively. In the placebo group, the dependent variable (GHBP after 6 months) could be predicted (adjusted $R^2 = .83$) from baseline GHBP only (Table 4). However, after extraction of the variance in baseline GHBP, the partial correlations of body fat mass ($r = -.07$) and IGFBP-3 ($r = .24$) were not significant ($P = .79$ and $P = .37$, respectively).

DISCUSSION

This study shows that 6 months of GH replacement therapy significantly increased the mean plasma levels of IGF-I and IGFBP-3 into the normal range. Moreover, plasma GHBP showed a significant time effect, although the changes in plasma GHBP were relatively small. In contrast, the placebo group showed no significant change in these variables. Recent studies have shown that the response of GHBP to GH treatment is variable in adults.[10,12-17,21] Stepwise multiple regression analysis of relevant baseline factors showed that in the placebo group, plasma GHBP levels after 6 months could be predicted from baseline plasma GHBP only (adjusted $R^2 = .83$), indicating an unexplained variance in the GHBP response of 17%. In the GH-treated group, posttreatment GHBP levels could be predicted accurately by the combination of baseline plasma GHBP, body fat mass, and plasma IGFBP-3, leaving an unexplained variance of only 3%. In both groups, the variables age, gender, fat-free mass, or IGF-I did not contribute to the prediction of the GHBP response. From a practical point of view, we chose to predict GHBP levels after 6 months rather than the change in GHBP, because the latter value is based on the difference of two values (GHBP after 6 months and baseline GHBP), without knowledge of their absolute values, although mathematically, the results of both predictions will essentially be the same.

Table 4. Regression Analysis of GHBP After 6 Months of Placebo Therapy on Baseline GHBP (pmol/L)

Variable	Coefficient	Standard Error	t	P
GHBP	0.98	0.11	8.89	.000
Constant	−0.80	146.82	−0.005	.99

Source of Variation	df	Sum of Squares	Mean Squares	F	P
Regression	1	1,058,719.06	1,058,719.06	78.98	.000
Residual	15	201,084.70	13,405.65		

It is well known that GHBP, body fat mass, and IGFBP-3 at baseline vary markedly in adult GHD subjects. For example, baseline plasma GHBP levels in the GH-treated group were 700 to 1,960 pmol/L, whereas baseline body fat mass and IGFBP-3 were 5.6 to 44.9 kg and 0.7 to 3.9 mg/L, respectively. In our study, gender-related differences in baseline GHBP could be excluded, and there is no need to adjust baseline GHBP levels for differences in the baseline BMI and body fat mass between the GH replacement and placebo groups. However, in the placebo group, no correlation was observed between baseline body fat mass and GHBP changes after 6 months ($r = -.08$, $P = .75$), whereas in the GH-treated group, a highly significant correlation ($r = .82$, $P < .001$) was found. This indicates that only in the GH-treated group did body fat mass contribute to the prediction of GHBP after 6 months. The physiological significance of the regression model is that in both groups the GHBP level after 6 months is primarily set by the baseline level of GHBP. Furthermore, this study shows that in the GH-treated group, the combination of baseline GHBP and body fat mass (ln-transformed) increases the explained variance in posttreatment GHBP from 85% to 95%, and to 97% when IGFBP-3 is included. Thus, body fat mass and IGFBP-3 can be considered "fine-tuners" in the GHBP response to GH treatment.

For example, GHD patients with a relatively high body fat mass and low IGFBP-3 will show an increase in GHBP after GH therapy. Assuming that plasma GHBP reflects GH receptor activity,[1-3] an increase in posttreatment GHBP would imply an upregulation or increased density of GH receptors. For clinical practice, this would mean that a lower GH dosage can be used for these subjects. It is also possible that an increased plasma GHBP level may reduce GH availability, as it has been reported that GHBP acts as a competitor to the GH receptor for GH binding and prolongs the circulating half-life of GH.[3,4]

Recently, Johannsson et al[16] reported on 64 GHD adults treated with GH for 12 months in whom the individual GHBP response changes from− 250 to +200 pmol/L, which is about the same order of magnitude as the changes in GHBP observed in our study. Further, Johannsson et al[16] presented changes in body fat as a function of changes in GHBP, with body fat changes as a dependent variable. Therefore, based on their data, it is not possible to analyze the contribution of the baseline body fat mass to the GHBP response. Recently, we reported that the GHBP response to GH therapy in adult GHD subjects was partially determined by the amount of abdominal fat.[17] This finding is in line with the observation by Rasmussen et al[22] that plasma GHBP levels are elevated in obesity, a condition associated with low GH secretion.[21,23] Fisker et al[18] reported that in healthy non-obese adults, serum GHBP levels seem to be determined by abdominal fat mass rather than by indices of GH status. In this study, we did not specifically measure the baseline abdominal fat mass with computed tomography or magnetic resonance imaging, because, as a first approximation, we aimed to investigate simple baseline variables for prediction of the GHBP response in clinical practice. However, all participating GHD patients in this study were centrally obese.

This study shows that a post hoc analysis of baseline values predicting the posttreatment response of GHBP in adult GHD

syndrome generates an accurate and reliable linear regression model that fits the experimental data extremely well by the combination of baseline GHBP, body fat mass, and IGFBP-3. The variations in body fat mass and IGFBP-3 among adult GHD subjects explain the reported variable response of GHBP to GH replacement therapy. The small but significant contribution of IGFBP-3 to the GHBP response in the GH-treated group needs further study for confirmation.

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