

*Research paper*

## Changes in prothrombin and activated partial thromboplastin time during replacement therapy with human recombinant growth hormone in growth hormone deficient adults

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### ABSTRACT

**BACKGROUND:** In rodents, Growth Hormone (GH) has been shown to stimulate coagulation parameters, including Prothrombin Time (PT), activated Partial Thromboplastin Time (aPTT) and vitamin K dependent coagulation factors. However, there are no reports on the influence of GH replacement therapy on global coagulation tests in Growth Hormone Deficiency (GHD). **OBJECTIVE:** The aim of this study was to investigate the effects of GH administration on basic coagulation parameters: PT, aPTT and fibrinogen concentrations in adult GHD patients before and during one year of GH replacement. **DESIGN:** Twenty-one adult patients with severe GHD (mean age  $\pm$  SE:  $38.6 \pm 2.8$  years) were included in this hospital based, prospective, interventional study. All patients were treated with rhGH for 12 months (GH dose: 0.4 mg/day for male and 0.6 mg/day for female patients). IGF-1 concentrations were determined using RIA-INEP kits. Basic coagulation tests, i.e. aPTT and fibrinogen concentrations, were measured before and after 3, 6 and 12 months of treatment with rhGH. Control values were obtained from fourteen "healthy" subjects matched by age, sex and body mass index (BMI). **RESULTS:** At baseline, we observed no significant differences in PT, aPTT and fibrinogen values between GHD and healthy subjects. IGF-1 concentrations increased significantly within 3 months of GH therapy ( $8.2 \pm 1.5$  vs.  $24.2 \pm 2.9$  nmol/l,  $p < 0.05$ ) and remained stable thereafter. A significant increase in PT values, which was more pronounced in female subjects, was noted after 6 and 12 months of treatment with GH. aPTT values increased significantly after 12 months of treatment only in male patients ( $28.8 \pm 4.6$  vs.  $39.7 \pm 2.1$  s.;  $p < 0.05$ ). No significant changes in fibrinogen concentrations were found during the study. **CONCLUSIONS:** Twelve months of GH replacement therapy led to a significant increase in PT and aPTT values in adult GHD patients, while fibrinogen concentrations did not change. Changes in PT were more pronounced in female GHD patients, while an increase in aPTT values was observed only in male patients with GHD. The clinical significance of these changes needs further evaluation.

**Key words:** Coagulation parameters, Growth hormone therapy, Growth hormone deficiency

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*Received 28-05-06, Revised 30-06-06, Accepted 03-07-06*

## INTRODUCTION

Previous studies have demonstrated that GH influences intrinsic and extrinsic coagulation pathways and synthesis of vitamin K dependent coagulation factors in rats.<sup>1,2</sup> On the other hand, data about the effects of GH therapy on coagulation factors in humans are scarce. Increase in circulating levels of F VIII and von Willebrand factor have been observed following the acute administration of GH to GH deficient children.<sup>3</sup> Johansson et al. showed decrease in tissue plasminogen activator (t-PA) concentrations and plasminogen activator inhibitor (PAI-1) activity in adult GH deficient (GHD) patients during GH replacement therapy.<sup>4</sup> However, there are no reports on the influence of GH replacement therapy on global coagulation tests in GHD subjects. Prothrombin Time (PT) and activated Partial Thromboplastin Time (aPTT) are basic coagulation tests which measure integrated actions of the majority of coagulation factors in extrinsic and intrinsic pathways of blood coagulation cascade. In our study we investigated the effects of one year GH replacement therapy on basic coagulation parameters: PT, aPTT and fibrinogen concentrations in twenty-one adult GHD patients, and compared them with fourteen "healthy" control subjects matched for age and BMI.

## SUBJECTS AND METHODS

The study was performed according to the Declarations of Helsinki. Written informed consent from all patients and approval from the local ethics committee were obtained. Twenty-one patients (11 females and 10 males, mean age  $\pm$  SE:  $38.6 \pm 2.8$  years, range 21-61) with biochemically proven GHD were

treated for 12 months with rhGH. The diagnosis of GHD was confirmed by two tests, i.e. ITT (peak GH response  $< 3 \mu\text{g/l}$ ) and GHRH+GRF-6 test (peak GH response  $< 10 \mu\text{g/l}$ ). Seventeen patients had undergone surgery (10 transcranial, 7 transsphenoidal). Eight patients were operated on for nonfunctioning pituitary adenoma (8/17), three for craniopharyngeoma (3/17), three for macroprolactinoma resistant to dopamine agonists (3/17), one for suprasellar astrocytoma (1/17), one for histiocytosis X (1/17) and one for Cushing's disease (1/17). In nine of these patients, surgery was followed by irradiation. One patient had idiopathic GHD. The mean period of time  $\pm$  SE after the initial diagnosis and/or therapy was  $10.1 \pm 1.7$  years (range 2-27). All patients were on continuous replacement therapy for other pituitary hormone insufficiencies before treatment with hrGH was initiated. Sixteen patients were treated with hydrocortisone replacement, fifteen with l-thyroxin, eighteen with sex steroids, five with dopamine agonists and three with desmopressin. Fourteen age, sex and BMI matched controls were also studied (Table 1). There were no smokers among the investigated subjects. All subjects were free of the diseases and medications which are known to affect PT and aPTT.

### GH treatment regimen

The initial dose of rhGH (Norditropin®, Novo Nordisk, Denmark) was: 0.6 mg/day for female and 0.4 mg/day for male patients, adjusted according to circulating IGF-1 levels after 4 weeks of treatment.

### Methods

Blood samples were taken after an overnight fast

**Table 1.** Clinical characteristics of investigated subjects. Mean value ( $\bar{x}$ ), standard error (SE), (Range)

Clinical characteristics	GHD patients			Control subjects		
	Female (N=11)	Male (N=10)	Total (N=21)	Female (N=8)	Male (N=6)	Total (N=14)
Age (yrs)	33.1 $\pm$ 3.1 (24-49)	39.7 $\pm$ 4.9 (21-61)	38.6 $\pm$ 2.8 (21-61)	34.6 $\pm$ 5.4 (19-52)	41.8 $\pm$ 7.4 (33-56)	37.3 $\pm$ 3.5 (19-56)
BMI (kg/m <sup>2</sup> )	26.3 $\pm$ 1.7 (20.4-35)	26.3 $\pm$ 1.4 (21.4-33.2)	26.4 $\pm$ 2.2 (20.4-35)	24.6 $\pm$ 1.6 (20-29)	27.1 $\pm$ 0.8 (24-30)	25.4 $\pm$ 3.0 (20-30)
Duration of the primary disease (yrs)	11.7 $\pm$ 1.5 (6-27)	8.7 $\pm$ 2.1 (2-21)	10.1 $\pm$ 1.7 (2-27)	0	0	0

from the cubital vein before and after 3, 6 and 12 months of rhGH therapy. For the coagulation studies, venous blood samples were collected in tubes containing 3.2% buffered sodium citrate (volume of blood: volume of citrate=9:1) and centrifuged at 3000g for 10 min. After separation of platelet poor plasma, coagulation tests were immediately performed using the ACL machine (Instrumentation Laboratory, Milan, Italy). PT and fibrinogen were determined using thromboplastin from human placentas (Thromborel S, Dade-Behring, Germany), while aPTT was measured using liquid silica as activator of intrinsic pathway of coagulation (Liquid silica, Instrumentation Laboratory, Milan, Italy). Laboratory reference range for fibrinogen concentration was 2-4 g/L. The values of PT expressed in seconds have been converted to percents of normal by using a reference curve which was established by serial dilutions of calibration plasma (Standard human plasma, Dade-Behring, Germany). Laboratory reference range for PT was 75-140%. The values of aPTT have been expressed in seconds(s); laboratory reference range 25-37s. IGF-1 concentrations were measured by commercial RIA kit (INEP, Zemun, Yugoslavia) and expressed in nmol/L. Normal

range for adults <40 years: 13-45 nmol/l, and for those >40 years: 9-30 nmol/l.

### Statistical analysis

We used the Wilcoxon paired test to compare patients before and during replacement with rhGH. The Mann-Whitney U-test was used to compare the patients with the healthy subjects. To investigate sex-related differences in coagulation parameters and IGF-1 levels we used the student's t-test. The resulting values are shown as mean±SE. The value considered statistically significant was  $p \leq 0.05$ .

## RESULTS

Initially, our patients had significantly lower levels of IGF-1 compared with controls (Table 2). An increase in IGF-1 levels was observed within 3 months of treatment (mean±SE: 8.2±1.5 vs. 24.2±2.9 nmol/l;  $p < 0.01$ ) with no significant additional increase thereafter (Table 2).

Before treatment, our patients and controls had normal mean fibrinogen, PT and aPTT values. No significant differences were found in values of coagulation parameters between patients and controls,

**Table 2.** Coagulation variables and IGF-1 levels of investigated subjects. Mean value ( $\bar{x}$ ), standard error (SE), (Range)

	Fibrinogen (g/l)	PT (%)	aPTT (s)	IGF-1 (nmol/L)
Control group	3.3±0.1 (2.9-4.5)	104.3±1.6 (92-113)	32.4±1.4 (27.8-36.1)	21.7±1.8 (14.6-36.7)
Female	3.5±0.2 (3.0-4.5)	105.6±1.3 (95-113)	32.6±1.2 (25-36.1)	22.8±3.7 (16.1-36.7)
Male	3.2±0.5 (2.9- 4.1)	100.8±1.0 (92-111)	31.1±1.1 (27.8-33)	20.2±2.0 (14.6-26.5)
GHD (at baseline)	3.7±0.3 (2.1-6.7)	103.3±2.8 (86-128)	32.8±1.3 (18.4-42.1)	8.2±1.5† (1.9-23.5)
Female	3.8±0.3 (2.5-5.7)	107.5±4.2 (91-128)	32.7±1.3 (25.4-38.5)	10.1±2.3† (2.4-23.5)
Male	3.6±0.6 (2.1-6.7)	97.3±2.6 (86-107)	28.8±4.6 (18.4-42.1)	7.8±1.6 †(1.9-15.9)
GHD (3 months)	3.8±0.2 (2.6-5.7)	108.8±5.4 (78-181)	31.4±1.1 (23-39)	24.2±2.9** (7.9-49.3)
Female	3.9±0.3 (2.6-5.7)	114.4±8.8 (92-181)	30.9±1.4 (23-39)	28.8±4.4** (7.9-46.4)
Male	3.5±0.2 (2.7-4.6)	100.8±1.9 (89-109)	32.3±1.9 (25-38)	24.7±4.6** (10.7-49.3)
GHD (6 months)	3.6±0.2 (2.3-5.0)	117.1±6.1*† (92-166)	31.6±1.5 (22.9-41.4)	25.3±4.1** (2.6-75.0)
Female	3.7±0.3 (2.3-5.0)	120.7±9.0*† (88-166)	31.7±2.0 (22.9-40)	21.6±5.9** (2.6-75.0)
Male	3.6±0.3 (2.4-5.0)	111.9±7.5*† (89-187)	31.3±2.3 (24.1-41.4)	26.4±4.9** (8.8-33.9)
GHD (12 months)	3.7±0.1 (2.6-5.4)	126.3±9.1*† (88-212)	34.9±2.2 (25.4-50.4)	23.3±2.8** (7.0-60.1)
Female	3.7±0.3 (2.6-5.4)	133.4±11.5*† (88-212)	30.3±1.1 (25.4-34.7)	21.9±2.6** (7.0-60.1)
Male	3.8±0.2 (3.2-4.9)	108.6±4.8*† (92-127)	39.7±2.1* (31.1-50.4)	22.9±3.1** (9.9-50.3)

\* patients before vs. after therapy  $p < 0.05$ , \*\* patients before vs. after therapy  $p < 0.01$ , †patients vs. controls  $p < 0.05$

although the patients tended to have higher mean fibrinogen levels than the controls (Table 2).

After 6 and 12 months of GH replacement, a significant increase in PT values was observed in both male and female patients compared to values before treatment and the values of the control group ( $p < 0.05$ ; Table 2). Before and during treatment, PT values were always higher in female than in male patients, although not significantly.

No significant changes in aPTT values were observed during rhGH therapy. Significant, gender dependent prolongation of PTT was observed only in male patients after 12 months of treatment ( $p = 0.001$ ) (Table 2). Seven out of ten male patients (70%) achieved prolongation of aPTT above the upper limit of normal after 12 months of treatment. No significant correlations between changes in IGF-1 and PT and aPTT values were found.

Fibrinogen concentrations, although initially slightly higher in our patients than in the healthy subjects, remained unchanged during the entire treatment period.

## DISCUSSION

In the present study, no statistically significant differences in PT and aPTT values between patients and healthy controls were found, suggesting that GH is not necessary for baseline synthesis of factors of extrinsic and intrinsic coagulation pathways and maintenance of these values within the normal range. It has been suggested that GH deficiency leads to increased cardiovascular mortality which, according to our data, is not related to impairment of the clotting mechanism, as no differences in fibrinogen, PT and aPTT were found between GH deficient patients before GH treatment and normal controls. Therefore, other mechanisms may be responsible for increased cardiovascular morbidity and mortality in these patients.

We have also demonstrated, for the first time, sexual dimorphism of GH actions on the coagulation system in GHD patients. The stimulatory effect of GH replacement therapy on prothrombin time was more pronounced in females, while prolongation of aPTT was observed only in male GHD patients.

An increase in PT values during GH treatment was first observed in animal studies.<sup>1,2</sup> Negrev et al found significant increases in PT, F II, F VII and F X after GH administration to normal male rats pretreated with somatostatin, suggesting that the observed increases in PT values may be due to an increase in synthesis and/or gamma carboxylation of vitamin K-dependent coagulation factors in the liver.<sup>1</sup> Savendahl et al showed decreased PT and F VII in hypophysectomized female rats on conventional replacement and significant increase in PT, F VII and F IX in these animals during GH replacement.<sup>2</sup>

Ten out of our twelve female patients (83.3%) were on estrogen/gestagen replacement for secondary hypogonadism with the low dose pill (Cyclo Proginova). The stimulatory effects of oral contraceptives on blood coagulation have been well documented.<sup>5</sup> It has been shown that estrogen replacement after 3 months of treatment significantly increases prothrombin time and factors VII and X while the "intrinsic clotting" (aPTT) is not affected.<sup>6</sup> The role of various gestagen components on the coagulation system and the somatotrope axis is still controversial.

Estrogens have been found to exert antagonistic effects on GH action in peripheral tissues,<sup>7,8</sup> explaining the need for higher doses of GH in female patients.<sup>9</sup> Freyschuss et al. demonstrated induction of estrogen receptor by growth hormone and glucocorticoid replacement in primary cultures of rat hepatocytes, possibly suggesting similar effects of these hormones under *in vivo* conditions.<sup>10</sup> In the light of these findings, it can be hypothesized that the stimulation of the extrinsic coagulation pathway during GH replacement in female patients can be a result of estrogen and the GH mediated synergistic effect on synthesis of vitamin K dependent coagulation factors in the liver. It is of interest to note that our male patients had lower baseline levels of PT than the female patients and a lesser (11% vs. 20%), though significant, increase in final PT values, probably due to lack of the estrogen mediated effect of GH on PT values.

Although overall aPTT values did not change during GH replacement, we observed a significant increase in aPTT in male patients after 12 months

of treatment. Male patients had lower baseline aPTT values compared to female patients and apart from this there was no apparent explanation for this sex difference in the change of aPTT values after 12 months of treatment. Negrev et al (1995)<sup>1</sup> reported a significant increase in aPTT in healthy male rats treated with GH, but the nature of this change is not clear.

It appears that the extrinsic pathway of coagulation, reflected by PT, is more strongly affected in females, while changes in the intrinsic pathway and aPTT are predominant in male patients with GHD. These sex differences in coagulation parameters have not been reported before in GHD patients during GH replacement.

The results of our study suggest that GH plays a role in the regulation of the coagulation system in GHD adults. Although no adverse clinical effects of GH replacement therapy on blood coagulation have been reported so far, the observed shifts in the intrinsic and extrinsic pathways of blood coagulation during therapy with GH stress the importance of close monitoring of PT and aPTT, especially in the group of patients treated with GH and anticoagulant therapy for cardiovascular problems. Further studies are needed to elucidate the mechanisms of GH action on the coagulation system and the clinical implications of the data herein reported.

#### ACKNOWLEDGEMENTS

We gratefully acknowledge Mr. Predrag Radosevic of Novo Nordisk-Belgrade for his invaluable assistance and support. Novo Nordisk, Denmark generously supplied the GH.

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