Gonadotropin treatment increases homocysteine levels in idiopathic hypogonadotropic hypogonadism: an indirect effect mediated by changes in body composition

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Abstract

The main objective of the present study was to examine the alterations in plasma total homocysteine (tHcy) concentrations during a testosterone-deficient state and after gonadotropin treatment for 6 months in patients with idiopathic hypogonadotropic hypogonadism (IHH). Thirty-five newly diagnosed male patients with IHH (mean age 21.34 ± 1.53 years) and 29 age- and body mass index-matched healthy males (mean age 21.52 ± 1.77 years) were recruited into the study. Pretreatment levels of free testosterone (1.51 ± 0.66 pg/ml), estradiol (21.37 ± 4.37 pg/ml), FSH (0.91 ± 0.24 IU/l) and LH (1.25 ± 0.53 IU/l) were lower than controls (25.17 ± 3.06 pg/ml, 31.00 ± 4.96 pg/ml, 3.14 ± 1.62 IU/l and 4.83 ± 1.65 IU/l respectively) ($P < 0.001$). They increased significantly after treatment (18.18 ± 1.59 pg/ml, 27.97 ± 4.25 pg/ml, 2.41 ± 0.27 IU/l and 2.79 ± 0.19 IU/l respectively) ($P < 0.001$). Patients with IHH had lower tHcy levels than controls (10.14 ± 1.34 and 12.58 ± 2.29 μ mol/l respectively) ($P < 0.001$). Plasma tHcy concentrations increased significantly (12.63 ± 1.44 μ mol/l) after 6 months of treatment ($P < 0.001$). As compared with the controls, pretreatment levels of serum creatinine

(63.54 ± 13.01 vs 82.84 ± 16.69 μ mol/l), hemoglobin (12.98 ± 0.56 vs 13.83 ± 0.71 g/dl) and hematocrit (39.29 ± 2.01 vs $41.38 \pm 1.95\%$) were significantly lower ($P < 0.001$), and they increased significantly following treatment (80.24 ± 11.93 μ mol/l, 13.75 ± 0.49 g/dl and $41.26 \pm 1.78\%$ respectively) ($P < 0.001$). The pretreatment folic acid and vitamin B₁₂ levels were significantly higher in patients when compared with controls (14.87 ± 5.68 vs 12.52 ± 4.98 nmol/l, $P = 0.034$ and 289.75 ± 92.34 vs 237.59 ± 108.17 pmol/l, $P = 0.002$ respectively). They decreased significantly after treatment (11.29 ± 3.31 nmol/l and 228.51 ± 54.33 pmol/l respectively) ($P < 0.001$). The univariate and multivariate regression analysis results showed that only changes in creatinine, creatinine clearance, vitamin B₁₂ and folic acid were independently associated with changes in tHcy levels in patients with IHH. In conclusion, the increase in plasma tHcy concentrations following gonadotropin treatment seems to be largely independent of changes in androgen levels.

Journal of Endocrinology (2003) **179**, 35–39

Introduction

There is a consistent body of evidence from studies indicating that fasting plasma total homocysteine (tHcy) concentrations are higher in men than in women, which has led to the suggestion that sex steroid hormones may influence tHcy levels (Jacobsen *et al.* 1994, van der

Mooren *et al.* 1994). Moreover, plasma tHcy levels were decreased by the administration of ethinyl estradiol and anti-androgen to male transsexual subjects and increased by the administration of androgen to female transsexual subjects (Giltay *et al.* 1998). Recently, high plasma tHcy levels were reported in patients with polycystic ovary syndrome (PCOS), a state characterized by high levels of

endogenous testosterone (Yarali *et al.* 2001). Furthermore, Ebenbichler *et al.* (2001) have also suggested that intake of anabolic androgenic steroids, as used by bodybuilders, induces hyperhomocysteinemia.

The long-term effects of androgens on plasma tHcy concentrations in humans is not difficult to study because there are at least two hypogonadal syndromes in men who have testosterone deficiency and require hormone replacement therapy. However, to our knowledge, there have been no reports on the effect of gonadotropin treatment on tHcy concentrations in idiopathic hypogonadotropic hypogonadism (IHH). In the present study, therefore, we had the following two objectives: (1) to examine the alterations in fasting plasma tHcy concentrations during a testosterone-deficient state in patients with IHH; and (2) to find out whether there is any effect of gonadotropin treatment on tHcy levels in these patients.

Materials and Methods

Subjects

Thirty-five newly diagnosed male patients with IHH, and 29 sex-, age- and body mass index (BMI)-matched healthy raw recruits were enrolled in the study. The diagnosis of IHH was based on failure to undergo spontaneous puberty before 18 years of age and was confirmed by a decreased serum testosterone concentration below the normal range for adults, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels within or below the normal range, absence of a pituitary or hypothalamic mass lesion on computed tomography or magnetic resonance imaging, presence of a gonadotropin response to repetitive doses of gonadotropin-releasing hormone, normal smell test and normal karyotypes (46, XY).

None of the patients had hyposmia, anosmia or a family history of IHH. All patients had scrotal testes. All controls had a history of spontaneous puberty and their physical and biochemical findings were within the normal range. Patients and controls who reported a history of renal, hepatic and vascular diseases, diabetes mellitus, hypertension, hyperlipidemia, malignancies, anemia, hypothyroidism, intake of androgen, thiazide diuretics, phenytoin, carbamazepine, theophylline, nitrous oxide, metformin, niacin, penicillamine, methotrexate, vitamin B₆, vitamin B₁₂ and/or folic acid supplements, excessive coffee consumption, chronic alcohol intake and current smoking were excluded. Strenuous physical activity was not allowed before the collection of blood samples.

All patients and control subjects were informed about the aim and procedures of the study and gave their consent. The study was approved by the Ethical Committee of Gülhane Military Medical Academy.

Study design

Patients were treated with human chorionic gonadotropin (hCG; Profasi HP 2000; Serona SA, Aubonne,

Switzerland; containing 2000 IU hCG) and human postmenopausal gonadotropin (Pergonal; Serona SA; containing 75 IU FSH and 75 IU LH) three times a week for 6 months. Hormonal and biochemical data were assessed 6 months after therapy in all patients.

Fasting blood samples were collected from patients and controls between 0800 and 0830 h after overnight fasting. Post-treatment blood samples were drawn 7 days after the final injection of gonadotropin. Venous blood samples were centrifuged within less than 60 min, which is sufficient to prevent an increase in plasma homocysteine resulting from *ex vivo* generation of homocysteine by erythrocytes. Plasma was stored at -80°C until homocysteine was measured.

Assays

Plasma tHcy concentrations were measured by high performance liquid chromatography (HPLC; Millipore Corp., Waters Chromatography Division, Milford, MA, USA) (Araki & Sako 1987). The intra- and interassay coefficients of variation (CV) were 1.96% and 2.43% respectively. Vitamin B₁₂ and folic acid levels were measured by RIA with reagents from Diagnostic Product Corp. (Los Angeles, CA, USA). The intra- and interassay CV values were 3.73% and 4.79% for vitamin B₁₂ and 3.3% and 3.8% for folic acid respectively.

Complete blood count analyses were performed with an automatic hemocounter (Cell-Dyn 1700; Abbott, Santa Clara, CA, USA). Total plasma cholesterol, albumin and triglyceride were measured by an enzymatic calorimetric method with an Olympus AU 600 autoanalyzer using reagents from Olympus Diagnostics, GmbH (Hamburg, Germany). Serum creatinine levels were determined using a modified kinetic Jaffé method (Cook 1971).

Creatinine clearance was calculated according to the modified Cockcroft–Gault formula (Cockcroft & Gault 1976): $[140 - \text{age (years)}] \cdot \text{weight (kg)} \cdot (1.22)/\text{serum creatinine } (\mu\text{mol/l})$.

Serum FSH, LH, estradiol and prolactin (PRL) concentrations were measured by immunoradiometric assay with reagents from Radim Techland (Angleur, Belgium). The intra- and interassay CV values were 4.4% and 6.0% for FSH, 4.8% and 5.4% for LH, 4.4% and 6.0% for estradiol and 4.6% and 6.0% for PRL. Serum free testosterone concentrations were determined by a solid-phase ¹²⁵I RIA with reagents from Diagnostic Product Corp. The intra- and interassay CV values for free testosterone were 3.8% and 4.2%. Serum sex hormone binding globulin (SHBG) levels were measured by RIA with reagent from Radim Techland. The intra- and interassay CV values for SHBG were 2.4% and 2.9%. The normal ranges in our laboratory are 9.27–14.01 $\mu\text{mol/l}$ for tHcy, <15 IU/l for FSH, <20 IU/l for LH, <60 pg/ml for estradiol, 15–45 pg/ml for free testosterone and 9–38 nmol/l for SHBG. The upper limit for PRL is 12 $\mu\text{g/l}$.

Table 1 Clinical and laboratory features of patients with IHH and controls. Values are means $\pm$ S.D.

	Controls (n=29)	Pretreatment	IHH (n=35) Post-treatment	Controls vs IHH (pretreatment) (P)	Pre- vs post- treatment (P)
Parameters					
Age (years)	21.52 $\pm$ 1.77	21.34 $\pm$ 1.53		0.674 ^a	
BMI (kg/m ²)	20.48 $\pm$ 1.05	19.94 $\pm$ 2.65	21.00 $\pm$ 1.81	0.210 ^a	0.001 ^d
Hemoglobin (g/dl)	13.83 $\pm$ 0.71	12.98 $\pm$ 0.56	13.75 $\pm$ 0.49	<0.001 ^a	<0.001 ^c
Hematocrit (%)	41.38 $\pm$ 1.95	39.29 $\pm$ 2.01	41.26 $\pm$ 1.78	<0.001 ^a	<0.001 ^d
Creatinine (μ mol/l)	82.84 $\pm$ 16.69	63.54 $\pm$ 13.01	80.24 $\pm$ 11.93	<0.001 ^b	<0.001 ^d
Creatinine clearance (ml/min)	128.38 $\pm$ 23.56	153.80 $\pm$ 26.48	131.46 $\pm$ 23.44	<0.001 ^a	<0.001 ^c
Plasma albumin (g/l)	38.03 $\pm$ 1.15	37.69 $\pm$ 1.11	38.16 $\pm$ 1.25	0.385 ^b	0.125 ^c
Total cholesterol (mmol/l)	4.42 $\pm$ 0.85	4.63 $\pm$ 0.99	4.88 $\pm$ 0.86	0.539 ^a	0.069 ^c
Triglyceride (mmol/l)	1.09 $\pm$ 0.18	1.02 $\pm$ 0.40	1.04 $\pm$ 0.37	0.079 ^b	0.324 ^d
Free testosterone (pg/ml)	25.17 $\pm$ 3.06	1.51 $\pm$ 0.66	18.18 $\pm$ 1.59	<0.001 ^a	<0.001 ^d
FSH (IU/l)	3.14 $\pm$ 1.62	0.91 $\pm$ 0.24	2.41 $\pm$ 0.27	<0.001 ^b	<0.001 ^d
LH (IU/l)	4.83 $\pm$ 1.65	1.25 $\pm$ 0.53	2.79 $\pm$ 0.19	<0.001 ^a	<0.001 ^c
Estradiol (pg/ml)	31.00 $\pm$ 4.96	21.37 $\pm$ 4.37	27.97 $\pm$ 4.25	<0.001 ^a	<0.001 ^c
SHBG (nmol/l)	26.93 $\pm$ 5.80	40.44 $\pm$ 13.12	35.63 $\pm$ 8.63	<0.001 ^b	0.222 ^c
PRL (μ g/l)	6.24 $\pm$ 1.88	6.86 $\pm$ 1.22	6.84 $\pm$ 0.97	1.119 ^a	0.923 ^c
tHcy (μ mol/l)	12.58 $\pm$ 2.29	10.14 $\pm$ 1.34	12.63 $\pm$ 1.44	<0.001 ^a	<0.001 ^c
tHcy/creatinine	0.15 $\pm$ 0.03	0.16 $\pm$ 0.02	0.16 $\pm$ 0.01	0.141 ^a	0.177 ^c
Vitamin B ₁₂ (pmol/l)	237.59 $\pm$ 108.17	289.75 $\pm$ 92.34	228.51 $\pm$ 54.33	0.002 ^a	<0.001 ^c
Folic acid (nmol/l)	12.52 $\pm$ 4.98	14.87 $\pm$ 5.68	11.29 $\pm$ 3.31	0.034 ^b	<0.001 ^d

^aIndependent sample *t*-test; ^bMann–Whitney U test; ^cpaired sample *t*-test; ^dWilcoxon signed rank test.

Statistical analysis

All the statistical analyses were performed by using SPSS 10.0 (SPSSFW; SPSS Inc., Chicago, IL, USA) statistical package. Descriptive statistics are given as the arithmetic mean $\pm$ S.D. First we examined the normality assumptions for the variables by the Kolmogorov–Smirnov test. For the pairwise comparisons we used the paired sample *t*-test when the normality assumptions were held and the Wilcoxon signed rank test otherwise. For the comparison of two groups we used the independent sample *t*-test or the Mann–Whitney U test. Relations among changes in tHcy and the other parameters were investigated by linear regression analysis using the change in tHcy as the dependent variable and the change in sex steroids, vitamin B₁₂, folic acid, creatinine and creatinine clearance as independent variables. After the linear regression analysis, we used multiple regression analysis with a backward stepwise method using the change in tHcy and tHcy/creatinine ratio as dependent variables and changes in creatinine, testosterone, FSH, LH, estradiol, PRL, folic acid and vitamin B₁₂ as independent factors. In the last stage, we found that creatinine, creatinine clearance, vitamin B₁₂ and folic acid were statistically important for the model while the other factors were not. We also calculated the coefficient of determination (adjusted *R*²) for the final model. *P* values less than or equal to 0.05 were evaluated as statistically significant (Zar 1996, Dawson & Trapp 2001).

Results

Pre- and post-treatment clinical and laboratory characteristics and the results of comparisons are given in Table 1. BMI was slightly but not significantly (*P*=0.210) lower in untreated patients as compared with controls, whereas it increased significantly after treatment (*P*=0.001). Pretreatment levels of free testosterone, estradiol, FSH and LH were lower than controls (*P*<0.001). They increased significantly after treatment (*P*<0.001). However, creatinine clearance was higher than controls before therapy (*P*<0.001), whereas it decreased significantly (*P*<0.001) after treatment. SHBG was higher than controls before therapy (*P*<0.001), whereas it decreased slightly but not significantly (*P*=0.222) after treatment. PRL levels did not differ significantly between the groups.

As compared with the controls, pretreatment levels of serum creatinine, hemoglobin and hematocrit were significantly lower (*P*<0.001), and they increased significantly following treatment (*P*<0.001). There was no difference between patients and controls with respect to tHcy/creatinine ratio and the mean levels of albumin, total cholesterol and triglyceride.

The pretreatment folic acid and vitamin B₁₂ levels were significantly higher in patients when compared with controls (*P*=0.034 and *P*=0.002 respectively). They decreased significantly after treatment (*P*<0.001). A scatter plot of tHcy levels in the controls and patients before and after treatment is shown in Fig. 1. Patients with IHH

Figure 1 A scatter plot of tHcy levels ($\mu\text{mol/l}$) in the controls and patients before and after treatment.

had lower tHcy levels than controls ($P < 0.001$). However, plasma fasting tHcy concentrations increased significantly ($P < 0.001$) after 6 months of treatment (mean increment 24.56%).

The linear and multiple regression analyses in patients with IHH revealed that only changes in creatinine, creatinine clearance, vitamin B₁₂ and folic acid were independently associated with changes in tHcy levels in patients with IHH (Table 2). These significant associations were consistently present in a multivariate regression analysis.

Table 2 Linear and multiple regression results of the change in tHcy levels of patients with IHH

	Linear regression		Multiple regression	
	Intercept	P	Intercept	P
Factors				
Creatinine ($\mu\text{mol/l}$)	0.952	<0.001	0.328	<0.001
Creatinine clearance (ml/min)	-0.964	<0.001	-0.447	<0.001
Vitamin B ₁₂ (pmol/l)	-0.840	<0.001	-0.109	0.015
Folic acid (nmol/l)	-0.790	<0.001	-0.079	0.038
Adjusted R ²			0.984	

Discussion

The present study is the first, to our knowledge, to examine the effects of gonadotropin therapy on tHcy concentrations in patients with IHH. We have shown that the plasma fasting tHcy concentrations in patients with IHH were low during the testosterone-deficient state as compared with controls, whereas a significant increase was observed after gonadotropin treatment. In agreement with our findings, Giltay *et al.* (1998) reported that plasma tHcy levels increased after androgen administration to female (transsexual) subjects. Ebenbichler *et al.* (2001) also suggested that the intake of anabolic androgenic steroids induced acute hyperhomocysteinemia. Similarly, high plasma tHcy levels were reported in patients with PCOS (Yarali *et al.* 2001). In contrast, in a cross-over study, short-term supra-physiological doses of testosterone did not alter fasting plasma tHcy concentrations in 14 normal male weightlifters (Zmuda *et al.* 1997). However, as indicated by these authors, this study had several limitations. First, a longer treatment duration and larger sample size might be required to reveal a testosterone effect on tHcy levels. Secondly, all subjects in that study had normal testosterone levels.

Our study revealed that plasma tHcy concentration tended to increase with creatinine and with decreasing creatinine clearance, plasma levels of vitamin B₁₂ and folic acid. It seems likely, therefore, that creatinine, creatinine clearance and vitamin status are determining factors for plasma tHcy levels in patients with IHH. These results also suggest that neither sex hormones nor BMI have an important, at least directly, influence on tHcy levels in IHH. In our study, as well as in others (Andersson *et al.* 1992, Brattstrom *et al.* 1994), serum creatinine was positively associated with plasma tHcy concentrations. The kidney plays an important role in the metabolism of homocysteine (Arnadottir *et al.* 1996, Wollesen *et al.* 1999) and, as such, it would explain not only why renal failure is an important cause of hyperhomocysteinemia, but also why creatinine is one of the biochemical parameters that correlate best with tHcy levels. This is probably due to the requirement, in the synthesis of the precursor of creatinine (creatinine), of the donation of methyl groups formed in the transformation of methionine to homocysteine (Mudd & Poole 1975). In this context, since androgens play an important role in the maintenance of muscle mass (Bhasin *et al.* 1996, Katznelson *et al.* 1996), increased turnover of muscle and protein following gonadotropin treatment may be the cause of the increase in creatinine. Furthermore, creatinine clearance was negatively associated with plasma tHcy in patients with IHH. These findings are in agreement with other authors who found strong correlations between tHcy and creatinine clearance (Arnadottir *et al.* 1996, Wollesen *et al.* 1999). It is possible that gonadotropin treatment may affect kidney function and it may be an another underlying factor for raised plasma tHcy concentrations following treatment.

In the current study, as in others (Andersson *et al.* 1992, Selhub *et al.* 1993, Ueland *et al.* 1993, Jacobsen *et al.* 1994), both vitamin B₁₂ and folic acid were negatively associated with plasma tHcy. On the other hand, it has been shown that androgens modulate erythropoietin receptor expression in erythroid cells and that androgens can also directly modulate the hematopoietic system, since androgen receptors have also been shown in erythroid cells of the bone marrow, indicating that androgens may exert direct effects on a wide spectrum of bone marrow cell types via androgen receptor-mediated responses (Jockenovel *et al.* 1997). As expected, hemoglobin and hematocrit levels increased significantly after gonadotropin treatment. Since synthesis of homocysteine takes place in erythrocytes to a small extent (Malinow *et al.* 1994), increased red blood cell turnover may lead to elevated plasma tHcy levels.

Treatment with folic acid normalizes the basal plasma homocysteine concentration in hyperhomocysteinemic patients with vascular disease, and even markedly reduces normal plasma homocysteine concentrations in non-folate-deficient healthy subjects (Brattstrom *et al.* 1994). In contrast, high doses of vitamin B₁₂ have not been found to have any such homocysteine-lowering effect. Although we did not study whether there is any effect of supplementation of vitamins on plasma tHcy concentration during the gonadotropin treatment, prophylactic supplementation of folate during gonadotropin treatment may prevent an increase in tHcy levels.

In conclusion, our findings have indicated that the plasma fasting tHcy concentration in patients with IHH was low during the testosterone-deficient state as compared with the controls, and it increased significantly after gonadotropin treatment. On the one hand, our observations suggest that androgens may have indirect effects, secondary to anabolic effects, on plasma tHcy levels. On the other hand, further studies are needed to answer the question as to whether the increase in tHcy levels following gonadotropin treatment raises the risk of cardiovascular events.

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Received in final form 26 June 2003

Accepted 9 July 2003

Made available online as an

Accepted Preprint 21 July 2003