0021-972X/98/$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 83, No. 2
Printed in U.S.A.

Effects of Sex Steroids on Plasma Total Homocysteine Levels: A Study in Transsexual Males and Females

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ABSTRACT

Plasma total homocysteine (tHcy) levels are higher in men *vs.* premenopausal women, but it is not known whether this difference is related to sex steroids. The effects of cross-sex hormone administration on plasma tHcy levels were therefore investigated.

Plasma tHcy levels were measured at baseline and after 4 months of treatment in 17 male-to-female (M→F) transsexuals treated with ethinyl estradiol (100 μg/day), in combination with the antiandrogen, cyproterone acetate (100 mg/day), and in 17 female-to-male (F→M) transsexuals treated with testosterone esters (250 mg/2 weeks, im).

In M→F transsexuals, the plasma tHcy level decreased from geometric mean 8.2 μmol/L to 5.7 μmol/L ($P < 0.001$); and in F→M transsexuals, it increased from 7.7 μmol/L to 9.0 μmol/L ($P = 0.005$). In M→F transsexuals, changes in serum sex hormone-binding globulin levels correlated negatively, and changes in plasma creatinine and albumin levels correlated positively, with changes in plasma tHcy levels. In F→M transsexuals, changes in serum 17β-estradiol levels correlated negatively, and changes in plasma creatinine levels correlated positively, with changes in plasma tHcy levels.

We conclude that tHcy levels decrease after estrogen + antiandrogen administration to male (transsexual) subjects, and levels increase after androgen administration to female (transsexual) subjects. These changes may be both primary and secondary to the anabolic/catabolic effects, as reflected by changes of creatinine and albumin levels after cross-sex hormone administration. (*J Clin Endocrinol Metab* **83:** 550–553, 1998)

MEN HAVE a higher risk of developing cardiovascular disease (CVD) than do premenopausal women. After menopause, the risk of CVD in women increases rapidly (1) and is reduced by estrogen replacement therapy (2). An elevated plasma level of total (free and protein-bound) homocysteine (tHcy) is a risk factor independent of other known risk factors for CVD (3–6). A sex difference in plasma tHcy levels has been found (7–10), with approximately 10–15% higher levels in men *vs.* women. There are indications that plasma tHcy is influenced by sex steroid hormones. Low plasma tHcy levels were found during pregnancy (11, 12), a state characterized by high levels of endogenous estrogens. Furthermore, in postmenopausal women, treatment with oral 17β-estradiol or conjugated estrogens resulted in a decrease of the plasma tHcy level by 11%, after 6 months (13, 14), which returned to base-line during a second half-year of treatment with conjugated estrogens (14). Supraphysiologic testosterone administration to normal males did not alter tHcy levels after 3 weeks, in a cross-over study (15). The aim of this study was to determine the effects on tHcy levels of cross-sex hormone administration to transsexuals undergoing sex-reassignment, creating a sex steroid milieu of the opposite sex.

Received March 24, 1997. Revision received September 5, 1997. Rerevision received October 17, 1997. Accepted October 21, 1997.

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Subjects and Methods

We included 17 male-to-female M→F transsexuals (median age: 28 yr; range: 18–40) and 17 female-to-male F→M transsexuals (median age: 22 yr; range: 16–34). M→F transsexuals were treated with ethinyl estradiol (100 μg/day; Lynoral, Organon, Oss, The Netherlands), in combination with the antiandrogen, cyproterone acetate (100 mg/day; Androcur, Schering, Weesp, The Netherlands). F→M transsexuals were treated with testosterone esters (Sustanon, Organon; 250 mg/2 weeks, im). There was no evidence of diabetes, hypertension, or other CVD in the subjects. None reported intake of hormones (such as oral contraceptives), medication known to affect sex steroid or lipid metabolism, or vitamin B_6, vitamin B_{12}, or folic acid supplements. The body mass index (BMI = weight/height^2) was determined. In 15 M→F transsexuals and 16 F→M transsexuals, the lean body mass (LBM) was estimated using bioelectrical impedance analysis (BIA 101/S, RJL Systems, Clinton Twp, Detroit, MI) (16). Before hormone administration, all F→M transsexuals had a regular menstrual cycle (28–31 days). Informed consent was obtained from all subjects, and the study was approved by the Ethical Review Committee of the Hospital Vrije Universiteit.

Fasting plasma tHcy levels were measured before and again after 4 months of cross-sex hormone therapy. At study entry, blood was drawn from F→M transsexuals between days 3 and 9 of the menstrual cycle during the follicular phase, and after 4 months within 5–9 days after the preceding testosterone injection. Samples were obtained between 0930 h and 1030 h and were immediately placed in ice and centrifuged at 3500 × *g* for 30 min at 4 C. After separation, plasma was stored at −70 C until analysis. To minimize the imprecision of the assay, samples from the same subject were analyzed in the same run. The plasma tHcy level was measured by using tri-*n*-butylphosphine as the reducing agent and ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulphonate as the fluorochromophore, followed by high-performance liquid chromatography with fluorescence detection (coefficients of variation: intraassay, 2.1%; interassay, 5.1%) (17).

Standardized RIAs were used to measure serum levels of 17β-estradiol (Double Antibody, Sorin Biomedica, Saluggia, Italy) and testosterone (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA). Se-

rum levels of LH and FSH were measured by immunometric luminescence assays (Amerlite, Amersham, UK). The lower limits of detection for LH, FSH, 17β-testosterone, estradiol, and were 0.3 IU/L, 0.5 IU/L, 90 pmol/L, and 1.0 nmol/L, respectively. Serum levels of sex hormone-binding globulin (SHBG) were measured by an immunoradiometric assay (Orion Diagnostica, Espoo, Finland). Plasma levels of creatinine, albumin, total cholesterol, low-density lipoprotein cholesterol, and liver enzymes (alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, and γ-glutamyl transpeptidase), as well as hemoglobin and packed cell volume, were assessed using standard laboratory methods. In 15 M→F transsexuals and 15 F→M transsexuals, the creatinine excretion rate was assessed using a 24-h urine collection. All laboratory measurements were carried out in blinded fashion, with respect to gender and hormone treatment status.

Statistical analysis

Because the distribution of plasma tHcy level was positively skewed, we used logarithmically transformed data in our analysis. Antilogarithms of the transformed means for plasma tHcy levels are presented (*i.e.* geometric means). The independent sample *t* test was used to compare tHcy levels between males and females at baseline. M→F and F→M transsexuals were analyzed separately. Statistical analysis of all variables was performed by the paired Student's *t* test. The Spearman's correlation coefficient was used to correlate proportional changes of tHcy levels with proportional changes of previously mentioned variables. Univariate and forward linear regression analysis was performed with the proportional change in plasma tHcy level as the dependent variable. If measurements were below the lower limit of detection, the value of that lower limit was used for statistical calculations (for LH, 0.3 IU/L; for FSH, 0.5 IU/L; for 17β-estradiol, 90 pmol/L; and for testosterone, 1.0 nmol/L). Two-sided $P < 0.05$ was considered statistically significant. The software used was SPSS for Windows 7.0 (SPSS Inc., Chicago, IL).

Results

Pretreatment values

All subjects were eugonadal at baseline by clinical and laboratory criteria. Lipoprotein distribution, liver enzymes, and plasma creatinine levels were within normal limits (Table 1). At study entry, the geometric mean of plasma tHcy in M→F transsexuals was 8.2 μmol/L (range: 4.6–36.7), and in F→M transsexuals, it was 7.7 μmol/L (range: 2.9–19.0). The sex difference of 6.5% was not statistically significant ($P = 0.66$). One M→F and one F→M transsexual had pretreatment levels higher than 15 μmol/L, which may be regarded as the upper normal limit (Fig. 1) (18).

Effect of cross-sex hormone administration

After estrogen + antiandrogen administration to M→F transsexuals, serum levels of testosterone decreased below the lower limit of detection. The administered ethinyl estra-

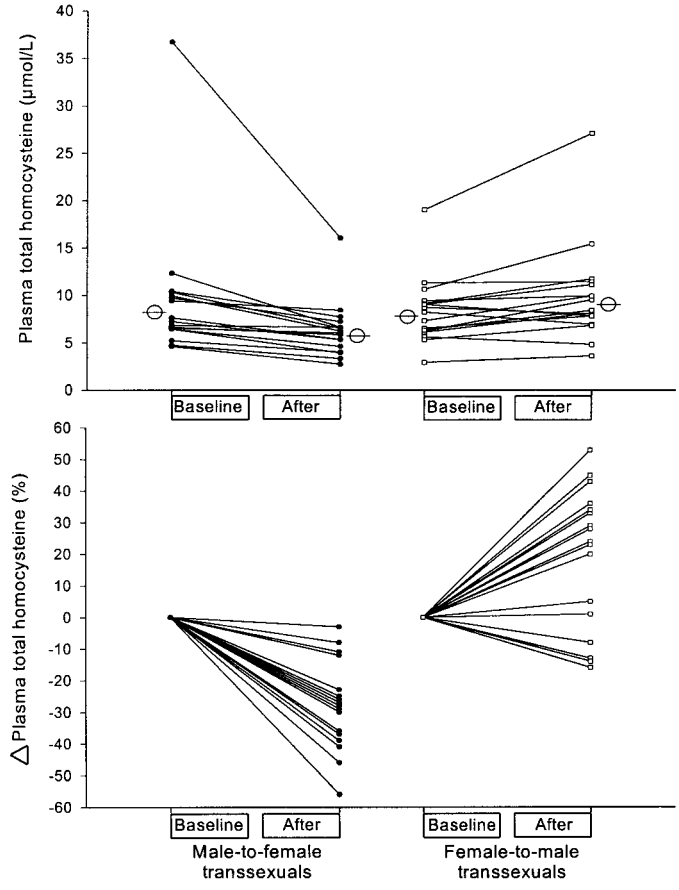

FIG. 1. Changes and proportional changes in plasma tHcy levels in 17 M→F transsexuals and 17 F→M transsexuals after 4 months of cross-sex hormone treatment, compared with baseline. Geometric mean values of the plasma tHcy level are identified by *open circles* and decreased with 30% ($P < 0.001$) in M→F transsexuals and increased with 17% ($P = 0.005$) in F→M transsexuals.

TABLE 1. Laboratory data before and after 4 months of cross-sex hormone treatment in 17 M → F and 17 F → M transsexuals

	Male-to-female transsexuals			Female-to-male transsexuals		
	Baseline	4 months	*P*-value	Baseline	4 months	*P*-value
17β-Estradiol (pmol/L)	97 ± 12	*		164 ± 60	132 ± 36	0.06
Total testosterone (nmol/L)	23.0 ± 6.5	1.0 ± 0.0	<0.001	1.6 ± 0.6	29.6 ± 11.2	<0.001
LH (IU/L)	3.3 ± 2.3	0.3 ± 0.1	<0.001	4.3 ± 2.2	2.7 ± 2.9	0.09
FSH (IU/L)	3.1 ± 3.1	0.5 ± 0.1	0.003	4.6 ± 1.4	3.7 ± 1.9	0.20
SHBG (IU/L)	36 ± 12	237 ± 53	<0.001	57 ± 30	26 ± 12	<0.001
Plasma creatinine (μmol/L)	86 ± 8	79 ± 9	<0.001	77 ± 9	85 ± 12	<0.001
Urinary creatinine excretion rate (mmol/24 h)	15.0 ± 4.1	15.3 ± 3.5	0.62	10.3 ± 2.8	12.1 ± 3.7	0.008
LBM (kg)	56.9 ± 9.3	56.6 ± 8.3	0.54	44.0 ± 6.2	48.1 ± 6.6	<0.001
BMI (kg/m^2)	21.0 ± 2.6	21.9 ± 2.5	0.005	21.9 ± 3.4	23.2 ± 3.2	<0.001
Albumin (g/L)	43 ± 3	36 ± 3	<0.001	43 ± 3	41 ± 3	0.08
Hemoglobin (mmol/L)	9.1 ± 0.6	8.0 ± 0.7	<0.001	8.3 ± 0.7	8.4 ± 0.5	0.61
Packed cell volume (L/L)	0.44 ± 0.04	0.37 ± 0.03	<0.001	0.40 ± 0.03	0.41 ± 0.03	0.17
Total cholesterol (mmol/L)	4.4 ± 1.2	4.7 ± 0.8	0.33	4.3 ± 0.9	4.1 ± 0.8	0.15
Low-density lipoprotein cholesterol (mmol/L)	3.0 ± 1.1	2.8 ± 0.4	0.32	2.6 ± 0.9	2.7 ± 0.9	0.66

Data are mean ± SD. *, M → F transsexuals were treated with ethinyl estradiol, which cannot be detected in conventional 17β-estradiol assays.

diol could not be detected by the assay used for measuring 17β-estradiol. After testosterone administration to F→M transsexuals, serum levels of testosterone increased markedly, whereas serum 17β-estradiol levels did not decrease substantially. However, in three subjects, the serum 17β-estradiol levels decreased below the lower limit of detection (Table 1).

In M→F transsexuals receiving estrogens + antiandrogens, plasma tHcy levels decreased from geometric mean 8.2 μmol/L (range: 4.6–36.7) to 5.7 μmol/L (range: 2.7–16.0), a mean decrease of 30% ($P < 0.001$). In F→M transsexuals receiving androgens, plasma tHcy levels increased from geometric mean 7.7 μmol/L (range: 2.9–19.0) to 9.0 μmol/L (range: 3.6–27.1), a mean increase of 17% ($P = 0.005$, Fig. 1). Proportional changes between plasma levels of tHcy correlated negatively with proportional changes in SHBG levels in M→F transsexuals ($r = -0.65$, $P = 0.008$), and with proportional changes in 17β-estradiol levels in F→M transsexuals ($r = -0.51$, $P = 0.04$), which remained significant after exclusion of the three subjects with estrogen levels below the lower limit of detection.

Effects of cross-sex hormone administration on other factors correlated with homocysteine levels

In M→F transsexuals, plasma levels of albumin, creatinine, hemoglobin, and packed cell volume significantly decreased. In F→M transsexuals, the plasma creatinine levels, the creatinine excretion rate, the LBM, and the BMI increased. None of the plasma creatinine levels were above the upper normal limit. Values of liver enzymes all remained within the normal range (Table 1).

The proportional change in plasma tHcy levels correlated with proportional changes in plasma creatinine and albumin levels in M→F transsexuals ($r = 0.55$, $P = 0.02$; $r = 0.59$, $P = 0.01$, respectively), and with proportional changes in plasma creatinine levels in F→M transsexuals ($r = 0.86$, $P < 0.001$) (Fig. 2); no correlations were found with the proportional changes in urinary creatinine excretion rate, LBM, BMI, hemoglobin, packed cell volume, plasma total cholesterol, and low-density lipoprotein cholesterol.

To further examine these relationships, we performed forward linear regression analysis, with the proportional change in plasma tHcy level as the dependent variable and proportional changes in previously mentioned variables as potential independent variables. In M→F transsexuals, the change in plasma albumin was the only correlate of plasma tHcy level (partial $r = 0.61$, $P = 0.02$), whereas the change in plasma creatinine was the only correlate in F→M transsexuals (partial $r = 0.83$, $P < 0.001$). Strikingly, after controlling for proportional change in plasma creatinine level, the effects of testosterone or estrogen + antiandrogen administration on tHcy levels disappeared in a linear regression model using all 34 subjects.

Discussion

A substantial reduction in plasma tHcy levels was found in male subjects after estrogen + antiandrogen administration. Because of simultaneous administration of antiandrogens, the observed effects may not be ascribed solely to estrogen administration. Conversely, a significant increase in plasma tHcy levels after testosterone administration was found in female subjects. This may have implications for the use of androgens in the treatment of hypoandrogenic males and for their growing use as a substitution therapy in aging males (19) or as a contraceptive agent in reproductive males (20). There have been reports of young weight lifters, using androgens, who developed myocardial infarction and stroke (21, 22).

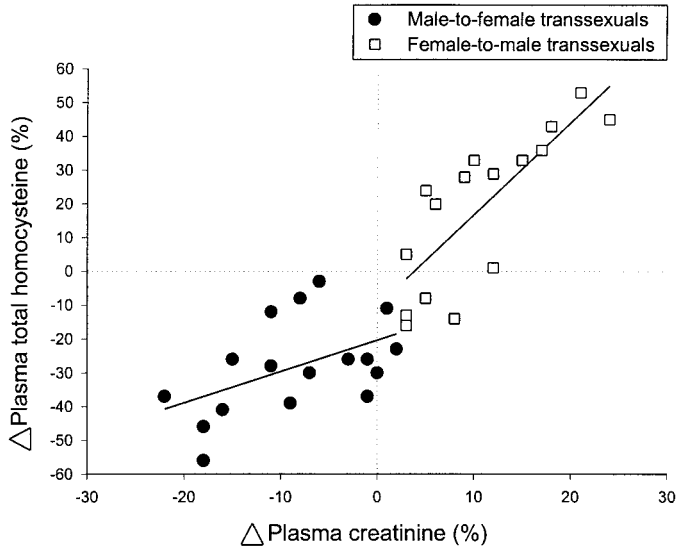

FIG. 2. Scatter plots, showing the correlation between the proportional change in plasma creatinine levels and proportional change in plasma tHcy levels in M→F transsexuals ($r = 0.55$, $n = 17$, $P = 0.02$) and in F→M transsexuals ($r = 0.86$, $n = 16$, $P < 0.001$). For M→F transsexuals in a regression model, the constant coefficient was −20% (95% confidence interval: −30% to −10%; $P = 0.001$).

Besides possible direct effects of the administered sex steroids on homocysteine metabolism, there also may be changes in plasma tHcy levels secondary to other biological effects of these steroids. First, deficiencies of vitamin B_{12} and folate lead to markedly increased plasma tHcy levels (18, 23). Oral contraceptives, containing ethinyl estradiol, are known to decrease serum concentrations of vitamin B_{12} and folate (24, 25). Levels of these vitamins were not determined in this study, because ethinyl estradiol was expected to lower tHcy levels (13, 14). Estrogen-induced effects on these vitamins should, if anything, have led to an increase of tHcy levels, but the opposite was observed. Second, plasma tHcy levels are related to protein turnover and muscle mass (26). In two studies, the sex difference of plasma tHcy levels disappeared after adjustment for plasma creatinine level (8, 9). This may be explained by the larger creatine synthesis and larger muscle mass in men, compared with women (27). Accordingly, we found that plasma creatinine was a strong correlate of the plasma tHcy level in both M→F and F→M transsexuals. An explanation for this could be that homocysteine production occurs in direct conjunction with creatine-creatinine synthesis (27). Renal failure, known to increase the plasma tHcy level (18, 28), did not occur in our subjects. Therefore, sex steroid-induced changes in plasma tHcy levels could conceivably be explained by their anabolic/catabolic effects.

Third, a large fraction (about 70%) of homocysteine is protein-bound, mainly by albumin (29), which was lowered during estrogen administration in M→F transsexuals. We did find a correlation between the change in plasma albumin and the change in plasma tHcy level in M→F transsexuals. Besides dilutional (12) or catabolic effects, a decrease in the plasma tHcy level could be a direct result of a reduction of albumin-bound homocysteine in M→F transsexuals. However, this seems unlikely, because the binding capacity of albumin for homocysteine is high.

Fourth, androgens have anabolic effects on erythropoiesis (30). To a small extent, synthesis of homocysteine takes place in erythrocytes (31), a potential source of homocysteine. However, there was no correlation between changes in hemoglobin or packed cell volume and changes in plasma tHcy levels. Anabolic steroids can induce liver-damaging side effects (32) and the enzyme [betaine]-homocysteine methyltransferase, necessary for the salvage of homocysteine to methionine, is mainly confined to the liver (18, 26). In our subjects, there were no changes in values of liver enzymes in response to cross-sex hormone administration.

Our findings provide support for indirect effects of sex steroids on plasma tHcy levels, secondary to anabolic/catabolic effects. However, primary hormonal effects can certainly not be excluded. M→F transsexuals who had no change in plasma creatinine level showed a decrease in plasma tHcy levels of 20%, suggesting a direct estrogenic effect on plasma tHcy levels (Fig. 2). Further, significant correlations were found in F→M transsexuals between changes in tHcy and 17β-estradiol levels and in M→F transsexuals between changes in tHcy and SHBG levels.

This study shows that plasma tHcy levels decrease after estrogen + antiandrogen administration to male subjects, and increase after androgen administration to female subjects. These changes may be direct or indirect through anabolic/catabolic effects. The sex difference in plasma tHcy levels thus seems related to their differences in sex steroid milieu.

Acknowledgments

We are indebted to J. A. J. Megens and Dr. P. J. M. van Kesteren for assistance in the logistics of this study; to C. Jacobs (Department of Clinical Chemistry) for measurement of plasma tHcy levels; and to Prof. Dr. A. J. M. Donker (Department of Internal Medicine, Hospital of the Vrije Universiteit), Prof. Dr. A. G. Smals, and Dr. G. H. Boers (Department of Endocrinology, University of Nijmegen) for their valuable comments.

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