Influence of exogenous oestrogen or (anti-) androgen administration on soluble transferrin receptor in human plasma

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Abstract

The objective of this investigation was to study the effects of sex steroids on levels of haemoglobin (Hb) and haematocrit (Hct) and to analyse whether these effects can be related to levels of the soluble transferrin receptor (sTfR), a marker of erythropoietic activity. Nineteen male-to-female transsexuals were randomly assigned to either oral ethinyl oestradiol (EE) ($n=12$) or transdermal 17β-oestradiol (E2) ($n=7$); both treatments included the anti-androgen cyproterone acetate (CA). Six male-to-female transsexuals were treated with CA only. Fifteen female-to-male transsexuals were treated with i.m. testosterone esters. The Hct, and levels of Hb, IGF-I, GH and sTfR were measured before and after 4 months of hormone administration. Androgen administration significantly increased the sTfR concentration by 31·5% ($P=0·008$) and increased levels of Hct, Hb and IGF-I. Both regimens of CA with oral EE and transdermal E2 reduced plasma testosterone similarly to castrate values and decreased Hb and Hct. The CA+oral EE combination induced a decrease in sTfR of 19·0% ($P=0·002$) which was not the case with CA+transdermal E2 ($P=0·27$). This cannot be explained by the profound decline in plasma testosterone which was similar with both regimens, but this difference could be related to the different effects of the two regimens on plasma IGF-I. This assumption is supported by the positive correlation that was found to exist between plasma sTfR and IGF-I after the interventions ($P<0·05$).

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Introduction

Men have higher haematocrit (Hct) and haemoglobin (Hb) concentrations than women (Morris *et al.* 1956), in all likelihood due to their higher plasma testosterone. Men who are hypogonadal have lower than normal Hct and Hb concentrations and testosterone treatment increases those variables to normal (Tenover 1992, Jockenhovel *et al.* 1997). In support of a role of testosterone is the observation that, in hypogonadal men, administration of transdermal testosterone dramatically increases Hct, from mildly anaemic to midnormal within 3 months (Snyder *et al.* 2000). Also, in healthy young men changes in circulating testosterone concentrations induced by gonadotrophin-releasing hormone (GnRH) agonist and testosterone administration are associated with testosterone dose- and concentration-dependent changes in Hb (Bhasin *et al.* 2001). The effects of testosterone on the Hct have been documented in several small and short-term studies, as reviewed by Hajjar *et al.* (1997). The mechanisms by which androgens exert these effects have not been elucidated. It has been suggested that androgen action on erythropoiesis is mediated by a nuclear androgen receptor (Claustres & Sultan 1988).

Administration of testosterone has been successfully used for the treatment of refractory anaemia in males (Piedras *et al.* 1998). It has been postulated that the administration of androgens to man and laboratory animals results in an increase in plasma erythropoietin activity. However, it has also been shown in recent years that in androgen-induced erythrocytosis, there is no increase in erythropoietin levels (Dickerman *et al.* 1998, 1999). Induction of androgen deprivation with a luteinizing hormone-releasing factor (LHRH) agonist did not result in changes in serum erythropoietin levels (Weber *et al.* 1991). Interestingly, there is no difference in serum erythropoietin levels between men and women, even though there is a significant difference in their testosterone and Hb levels (Miller *et al.* 1985). So, it seems that the androgen-mediated increases in Hb levels and Hct are not exclusively mediated by erythropoietin, and testosterone may have a direct effect on bone marrow stem cells (Shahidi 1973, Krabbe *et al.* 1978, Mooradian *et al.* 1987, Krauss

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et al. 1991). Our study monitored the effects of sex steroids on Hb and the Hct in transsexuals undergoing cross-sex hormone administration, making use of a quantitative assay of bone marrow erythropoietic activity, the soluble transferrin receptor (sTfR).

Iron transport in the plasma is carried out by transferrin. The interaction with a specific membrane receptor, the TfR allows iron to be included in cells. In both animal and human serum a soluble form of the TfR (sTfR) has been identified (Beguin 2003). Marrow erythropoietic activity appears to be the most important determinant of sTfR levels (R'Zik & Beguin 2001). In situations characterized by diminished erythropoietic activity, sTfR levels are decreased. When erythropoiesis is stimulated sTfR is increased. Measurements of sTfR are useful for monitoring the erythropoietic response to various forms of therapy. Predicting an early therapeutic response by measuring sTfR is possible when changes in Hb are not yet apparent (Beguin 2003).

To our knowledge, this is the first paper describing the effects of exogenous oestrogens and (anti-) androgens on the levels of the sTfR.

Materials and Methods

Subjects

We included 25 male-to-female (M→F) and 15 female-to-male (F→M) Caucasian transsexuals. Additional details of this study group can be found in Giltay *et al.* (2000). Psychological criteria for the diagnosis and treatment followed the guidelines provided by the Harry Benjamin International Gender Dysphoria Association (Walker *et al.* 1985). Nineteen M→F transsexuals were open-label-randomised to receive either oral ethinyl oestradiol (EE) (Lynoral, 100 μg/day; Organon, Oss, The Netherlands; $n=12$) or transdermal 17β-oestradiol (E2) (Estraderm TTS 100, 100 μg twice a week; CIBA-Geigy, Basel, Switzerland; $n=7$), both in combination with cyproterone acetate (CA) (Androcur, 100 mg/day; Schering, Berlin, Germany) which is a progestational compound with androgen receptor-blocking properties. Because the effects of the administration of CA alone on sTfR levels are unknown in men, we also studied six M→F transsexuals who received CA alone during the first 4 months of cross-sex hormone administration. F→M transsexuals were treated with testosterone esters (Sustanon, 250 mg/2 weeks, i.m.; Organon). All F→M transsexuals had had regular menstrual cycles (28–31 days) before cross-gender sex hormone administration. There was no evidence of iron deficiency, hypertension, cardiovascular disease, thromboembolism, diabetes mellitus or use of sex hormones in any of the subjects tested. Due to the availability of blood samples, the number of participants in this freezer study is smaller than in the study of Giltay *et al.* 2000 where 30 M→F transsexuals receiving CA+oral or transdermal oestrogens, 10 M→F transsexuals receiving CA alone, and 17 F→M transsexuals were described.

Smoking status (yes or no) and body mass index (BMI, weight/height^2) were also assessed. Informed consent was obtained from all subjects, and the study was conducted according to the principles of the Declaration of Helsinki and approved by the Ethical Review Board of the University Hospital Ghent and the Free University Amsterdam.

Assays

Each subject served as his or her own control, with samples drawn before and during hormone administration. In F→M transsexuals, blood was drawn at baseline between days 5 and 9 of the follicular phase of the menstrual cycle. During testosterone treatment, blood was drawn within 5–9 days after the previous testosterone injection. An intravenous catheter was placed in the antecubital vein of supine subjects after an overnight fast and 10 min of bed rest. Because of the travel time to the clinic, the time of blood sampling was between 0830 h and 1330 h. Within-subject time of sampling was, however, comparable before and after 4 months of hormone administration: mean 1055 h (95% confidence interval (CI), 1037–1112 h) versus mean 1037 h (95% CI, 1019–1055 h) respectively, $P=0{\cdot}10$. The mean intraindividual variation was 50 min (95% CI, 40–70 min). Blood was collected without a tourniquet into evacuated tubes (Diatube H CTAD, i.e. citrate, theophylline, adenosine and dipyridamole; Becton Dickinson, Rutherford, NJ, USA). Samples were immediately placed on ice and centrifuged at 3500 ***g*** for 30 min at 4 °C to obtain platelet-poor plasma. Plasma was separated and snap-frozen within 1 h and stored at −70 °C until analysis. The samples underwent one freeze–thaw cycle before serum sTfR, iron, transferrin and ferritin were measured. Serum sTfR was measured by a commercially available ELISA (R&D, Minneapolis, MN, USA). Serum ferritin was measured by the nephelometry method (Behring Nephelometer Analyzer; Dade Behring Marburg Co. Ltd, Marburg, Germany) following the manufacturer's instructions. Iron and transferrin were measured by standard methods (Modular; Roche). Standardized radioimmunoassays were used to measure serum levels of E2 (Double Antibody; Sorin Biomedica, Saluggia, Italy) and testosterone (Coat-A-Count; Diagnostic Products Corp., Los Angeles, CA, USA). Serum levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were measured by immunometric luminescence assays. Commercial assays were used for determinations of growth hormone (GH) (Immulite 2000; Diagnostic Products Corp.) and insulin-like growth factor (IGF-I) (extraction method, DSL-5600; Diagnostic System Laboratories, Webster, TX, USA). The effects of transdermal administration of E2 were compared with those of oral EE, both in combination with oral CA.

Table 1 Baseline characteristics of the study cohort

	M→F transsexuals					F→M transsexuals	
	Oral ethinyl oestradiol* plus CA (*n*=12)	Transdermal 17β-oestradiol* plus CA (*n*=7)	*P*†	CA** (*n*=6)	*P*‡	Testosterone-esters (*n*=15)	P§
Age (years)	31 ± 7	27 ± 5	0·144	32 ± 3	0·861	27 ± 6	0·093
BMI (kg/m^2)	23·1 ± 2·8	20·3 ± 2·0	0·035	21·5 ± 2·4	0·255	23·7 ± 4·4	0·113
sTfR (μmol/l)	18·4 ± 3·2	20·8 ± 6·8	0·326	24·2 ± 5·0	0·009	21·3 ± 5·4	0·632
Haematocrit (%)	0·42 ± 0·04	0·40 ± 0·02	0·400	0·44 ± 0·04	0·657	0·37 ± 0·02	<0·001
Haemoglobin (mmol/l)	9·1 ± 1·0	9·3 ± 0·5	0·652	9·2 ± 0·5	0·774	7·9 ± 0·5	<0·001
Serum iron (μg/dl)	129·5 ± 36·2	133·2 ± 50·5	0·855	181·2 ± 55·7	0·036	116·1 ± 57·0	0·160
Ferritin (ng/ml)	122·1 ± 74·8	154·3 ± 68·2	0·369	126·2 ± 75·2	0·919	55·8 ± 40·4	0·001
Transferrin (g/l)	2·57 ± 0·48	2·65 ± 0·33	0·701	2·46 ± 0·43	0·696	2·65 ± 0·46	0·583
IGF-I (ng/ml)	352·6 ± 119·6	386·9 ± 72·5	0·503	357·8 ± 120·5	0·936	390·5 ± 149·8	0·527
GH (ng/ml)	3·05 ± 4·75	1·35 ± 1·71	0·902	4·09 ± 4·40	0·679	4·56 ± 5·20	0·248

Data are means ± S.D. M→F, male-to-female; F→M, female-to-male. *randomised; **non-randomized; †*P* value by Student's *t*-test comparing males treated with oral ethinyl oestradiol versus transdermal 17β-oestradiol; ‡*P* value by Student's *t*-test comparing males treated with oral ethinyl oestradiol versus those treated with CA only; §*P* value by Student's *t*-test comparing all males and females.

Statistics

Most variables in the analysis turned out to be positively skewed. In order to meet the necessary model assumptions, a natural logarithmic transformation in these analyses was used for hormonal parameters and sTfR. All data are given as (geometric) means (± S.D. or 95% CI). Student's *t*-tests for independent samples or χ^2 tests were used to compare baseline differences. ANCOVA was used for intergroup comparisons after adjusting for possible confounders. Baseline values were correlated with the Pearson correlation coefficient.

An ANOVA for repeated measurements or a Student's *t*-test for paired samples was used to analyse the effects of cross-gender sex hormones. An ANCOVA was used to compare effects of different treatment regimens and to adjust for potential covariates. Proportional changes, after 4 months of cross-sex hormone administration were correlated with the Spearman correlation coefficient. If values were below the lower limit of detection, the value of that lower limit was used for statistical analysis (1·0 nmol/l for testosterone, 0·3 IU/l for LH and 0·5 IU/l for FSH). A two-tailed $P<0·05$ was considered statistically significant. The software used was SPSS 10·0 for Windows 8·0.

Results

Pre-treatment values

At baseline, all subjects were eugonadal by clinical and laboratory criteria. Iron deficiency as judged from serum iron, ferritin and transferrin serum levels was not present in any of the participants. Baseline characteristics are presented in Table 1. There was at baseline an expected difference between men and women for Hb, Hct and ferritin ($P<0·001$), but no significant difference for sTfR, iron or transferrin levels. In M→F transsexuals at baseline, there were no significant differences between the two groups except for BMI that was significantly ($P=0·035$) higher in males randomised to treatment with oral EE compared with males randomised to treatment with transdermal E2. Between M→F transsexuals treated with oral EE and M→F treated with CA only there was a difference in sTfR and serum iron levels at baseline (both $P<0·05$). There was no significant difference in sTfR between M→F transsexuals treated with transdermal E2 and M→F transsexuals treated with CA only.

Effects of sex steroid administration

In the group treated with CA alone, there were small but significant decreases in serum levels of testosterone and E2 ($P<0·05$) after 4 months treatment, and no changes in LH and FSH ($P=0·72$ and 0·22 respectively). Hb, Hct and sTfR levels did not change, while there was a trend for serum IGF-I to increase (P=NS). No significant relation between IGF-I and sTfR was established.

Oral and transdermal oestrogen administration, both with CA, decreased serum levels of testosterone (both $P<0·001$), LH ($P<0·001$ and $P=0·007$ respectively) and FSH ($P<0·001$ and 0·003 respectively). The combination of CA+EE administration (the latter not measured by the oestradiol assay used) suppressed endogenous oestradiol levels; whereas percutaneous administration of E2 increased plasma levels of E2 up to values typical of the midfollicular phase in women (Table 2).

In the group receiving CA+oral EE, Hb, Hct, IGF-I and sTfR levels decreased, whereas the increase in GH

Table 2 Laboratory data before and after 4 months of cross-gender sex hormone administration in M→F transsexuals, randomised for administration of oral ethinyl oestradiol in combination with CA or transdermal 17β-oestradiol in combination with CA

	M→F transsexuals (*n*=12), oral ethinyl oestradiol+CA			**M→F transsexuals (*n*=7), transdermal 17β-oestradiol+CA**		
	Baseline	4 months	*P*	Baseline	4 months	*P*
17β-oestradiol (pmol/L)	99·9 ± 33·4	24·7 ± 4·4	<0·001	82·0 ± 17·60	191 ± 152	0·121
Testosterone (nmol/L)	22·6 ± 6·5	1·0 ± 0·1	<0·001	22·1 ± 6·5	1·1 ± 0·2	<0·001
LH (IU/L)	3·2 ± 1·7	0·3 ± 0·0	<0·001	2·4 ± 1·1	0·4 ± 0·3	0·007
FSH (IU/L)	3·2 ± 1·5	0·5 ± 0·0	<0·001	2·0 ± 0·8	0·5 ± 0·0	0·003
Haematocrit (%)	0·42 ± 0·04	0·36 ± 0·02	<0·001	0·43 ± 0·02	0·39 ± 0·01	<0·001
Haemoglobin (mmol/l)	9·1 ± 1·0	8·0 ± 0·4	0·001	9·3 ± 0·5	8·6 ± 0·3	0·001
sTfR (μmol/l)	18·4 ± 3·2	14·9 ± 1·7	0·002	20·8 ± 6·8	19·3 ± 4·0	0·265
IGF-I (ng/ml)	352·6 ± 119·6	244·2 ± 100·7	0·034	386·9 ± 72·5	458·9 ± 78·3	0·004
GH (ng/ml)	3·05 ± 4·75	4·69 ± 4·04	0·415	1·35 ± 1·71	1·63 ± 2·38	0·412

Values are means ± S.D. Ethinyl oestradiol, which suppresses endogenous 17β-oestradiol, cannot be detected in conventional 17β-oestradiol assays.

was not significant. Soluble TfR levels correlated with IGF-I (r=0·643, P=0·024) at month 4. In the group receiving transdermal E2 and CA, there was a decrease in Hb and Hct after 4 months treatment (both P<0·001); the decrease in sTfR was not significant (P=0·27), while serum levels of IGF-I rose (P=0·040). There was a correlation between sTfR levels and IGF-I (r=0·821, P=0·023) at month 4.

After testosterone administration to F→M transsexuals, serum levels of testosterone increased markedly (P<0·001), whereas serum levels of E2, LH and FSH decreased (P=0·03, 0·01 and 0·005 respectively) (Table 3). The F→M transsexuals had significantly higher testosterone levels after 4 months of treatment, compared with the 3 groups of M→F transsexuals at baseline (P<0·001). Hb, Hct, sTfR and IGF-I rose significantly after testosterone administration in F→M transsexuals. There was no significant relation between IGF-I and sTfR in this group at month 4. However, the relation between IGF-I and sTfR at month 4 was confirmed when including all participants, both female and male (r=0·689, P=<0·01). Plasma iron concentrations were unaffected by the different hormonal treatments (data not shown).

Discussion

This study addressed the effects of sex steroids on Hb, Hct and the sTfR. The latter is a marker of marrow erythropoietic activity and is useful as an early indicator of diminished/increased erythopoietic activity (Beguin 2003). At baseline, males and females, in spite of their significant differences in plasma testosterone, Hb and Hct, did not show differences in values of sTfR. The possible interpretation of this observation is that differences in plasma testosterone may produce sex differences in values of Hb and Hct, but once baseline erythropoietic activity is stable, levels of sTfR are comparable in males and females; sTfR being an indicator of increases/decreases in erythropoietic activity.

A clear example of an effect of androgens was provided by testosterone administration to females. The dose of

Table 3 Laboratory data before and after 4 months of cross-gender sex hormone administration in F→M transsexuals receiving testosterone and in M→F transsexuals receiving CA only

	F→M transsexuals (*n*=15), testosterone			**M→F transsexuals (*n*=6), CA only**		
	Baseline	4 months	*P*	Baseline	4 months	*P*
17β-oestradiol (pmol/L)	176·8 ± 77·3	130 ± 34·6	0·03	77·3 ± 15·7	44·8 ± 17·7	0·023
Testosterone (nmol/L)	2·0 ± 0·8	34·6 ± 7·8	<0·001	20·3 ± 4·4	10·2 ± 7·3	0·029
LH (IU/L)	5·8 ± 3·6	2·6 ± 2·1	0·01	2·5 ± 1·2	2·6 ± 1·1	0·717
FSH (IU/L)	4·3 ± 1·0	2·9 ± 1·1	0·005	2·9 ± 2·3	2·6 ± 2·5	0·224
Haematocrit (%)	0·37 ± 0·02	0·40 ± 0·03	0·001	0·44 ± 0·04	0·42 ± 0·04	0·205
Haemoglobin (mmol/l)	7·9 ± 0·5	8·4 ± 0·9	0·016	9·4 ± 0·6	8·7 ± 1·0	0·234
sTfR (μmol/l)	21·3 ± 5·4	28·0 ± 8·4	0·008	24·2 ± 5·0	26·1 ± 3·9	0·350
IGF-I (ng/ml)	390·5 ± 149·9	446·5 ± 145·1	0·050	357·8 ± 120·8	433·6 ± 64·4	0·088
GH (ng/ml)	4·56 ± 5·20	5·18 ± 5·19	0·686	4·09 ± 4·35	2·10 ± 3·03	0·184

Values are means ± S.D.

Table 4 Overview of changes in levels of testosterone, IGF-I, haemoglobin (Hb) and sTfR between baseline and 4 months of cross-gender sex hormone administration

	Testosterone	IGF-I	sTfR	Hb
M→F transsexuals, oral EE+CA	↓	↓	↓	↓
M→F transsexuals, transdermal 17β-oestradiol+CA	↓	↑	=	↓
M→F transsexuals, CA only	↓	=	=	=
F→M transsexuals, parenteral testosterone esters	↑	↑	↑	↑

testosterone administered to females induced plasma testosterone levels in the high-normal or above-normal range for men and after 4 months increased Hb, Hct and sTfR, together with higher serum IGF-I levels (Table 4), but there was no demonstrable correlation between levels of IGF-I and sTfR. However, a reduction in circulating testosterone to approximately 50% of baseline by CA only in males – which also has antiandrogenic effects at the level of the androgen receptor – had no effect on Hb, Hct and sTfR values. A non-significant increase in serum IGF-I was seen in this group, with a non-significant decrease of serum GH levels. In this group, no correlation could be established between levels of IGF-I and sTfR.

The findings in males receiving CA+oestrogens were very remarkable. Both treatment regimens (CA+oral EE and CA+transdermal E2) equally suppressed plasma testosterone levels to castrate values and reduced values of Hb and Hct similarly, but only oral EE significantly reduced levels of sTfR. It should be noted that the effect of transdermal E2+CA on Hb and Hct showed a tendency to be smaller than in the other group receiving CA+oral EE, although not significantly. The difference in effect on sTfR between the two oestrogen regimens cannot be explained by their effects on plasma testosterone levels: both regimens decreased plasma testosterone similarly to castrate values. The quantitative difference in sTfR between oral and transdermal oestrogen may have been related to the different potencies of the two oestrogen regimens. EE is much more biopotent than E2. However, another mechanism to consider may be the impact of the route of oestrogen administration on the somatotropic axis. Previous studies have documented that metabolic effects of oral and transdermal oestrogens may differ, implicating hepatic mechanisms or effects on serum IGF-I in treatment with oral oestrogens (Chetkowski *et al.* 1986, De Lignieres *et al.* 1986, Lisset & Shalet 2003). Approximately 60% of orally administered EE is inactivated by the liver via a first-pass effect through the enterohepatic circulation. Orally administered EE (with a strong hepatic impact) compared with transdermally administered E2 (with less hepatic effects) might produce a different effect on the somatotropic axis. It is known that in post-menopausal women oral EE has a strong hepatic effect with impaired hepatic IGF-I production that causes increased GH secretion through reduced feedback inhibition (Ho *et al.* 2003). This was earlier also shown in the study of Van Kesteren *et al.* (1996) in transsexual persons. In the study by Ho *et al.* (2003) transdermal administration of E2 resulted in a slight increase in serum IGF-I but no change in mean 24 h GH levels. High-dose transdermal oestrogen (200 μg/day) in postmenopausal women did not affect basal levels of IGF-I in the study by Lissett and Shalet (2003). In our study, transdermal E2+CA resulted in a significant increase in IGF-I levels whereas oral EE+CA resulted in a significant decrease of IGF-I levels. CA was part of both oestrogen regimens. There were no demonstrable effects of CA only on Hb, Hct and sTfR, though a tendency of IGF-I to increase with a tendency of GH to decrease were noted. The most likely interpretation is that oral CA, a potent progestagen, increases hepatic IGF-I production with a negative feedback effect on pituitary GH production. The addition of transdermal E2 to CA probably had little or no additional effect on the increase of serum IGF-I observed with administration of CA only. This is consistent with earlier observations on transdermal E2 on the IGF-I/GH axis. In agreement with data from the literature, oral EE treatment may have counteracted the tendency to IGF-I increase which was seen with CA only administration. The significant decrease of IGF-I following administration of CA+EE substantiated this assumption.

Androgen therapy leading to supraphysiological plasma testosterone levels produced a stimulation of bone marrow erythropoietic activity, possibly through a direct effect of testosterone on erythropoiesis, but possibly in part mediated through IGF-I induction. The administration of CA only resulted in an approximately 50% reduction in plasma testosterone but had no effect on Hb, Hct or sTfR, possibly explained by the concomitant rise of IGF-I. There was no correlation between levels of IGF-I and of sTfR in this group. Transdermal E2 combined with CA had similar increasing effects on IGF-I levels as CA only, without a demonstrable effect on sTfR, but there was a correlation between levels of IGF-I and sTfR. The decrease in Hb and Hct must probably be ascribed to the profound fall in plasma testosterone. However, administration of oral EE combined with CA reduced sTfR levels significantly, with a correlation between levels of IGF-I and of sTfR. The effects of the decrease in testosterone and IGF-I levels may be responsible for the significant decrease in sTfR levels upon administration of CA with oral oestrogens, while after transdermal

oestrogens plus CA – in spite of a decline of plasma testosterone – there was no decrease of sTfR; this can probably be explained by the rise of IGF-I with the latter regimen. This speculation is further substantiated by the significant correlation of plasma sTfR and IGF-I following both oestrogen regimens.

A larger number of participants and the measurement of other potential confounders, such as erythropoietin levels, would have strengthened the interpretation of these data. This study paves the way for other larger studies that are needed to assess these interactions further. Evaluating the various effects in shorter intervals and for a longer period of time would be interesting in order to see if described effects do or do not persist.

In conclusion, our results show that profound alterations in plasma testosterone levels from male to female values, and vice versa, are associated with significant changes in Hb and Hct of the order of 10%. A reduction of plasma testosterone of the order of 50% had no impact on Hb and Hct. We tested whether these changes in Hb and Hct could be related to levels of sTfR, a marker of diminished/increased erythropoietic activity. Indeed, the increase in Hb/Hct upon testosterone administration was associated with an increase in sTfR, while there was no change in sTfR levels with lack of change of Hb/Hct upon administration of CA. The fall in Hb/Hct levels upon administration of CA combined with either oral EE or transdermal E2 had disparate effects on levels of sTfR: a decline with CA+oral EE, but no effect on levels of sTfR was observed with CA+transdermal E2. This disparity cannot be explained by the effects these two hormone regimes had on plasma testosterone. Earlier studies have indicated that administration of oral EE reduces plasma IGF-I, which is not the case with transdermal E2. In addition, indeed, in our study a correlation between plasma IGF-I and sTfR could be demonstrated after the intervention with the two modes of oestrogen administration and for the total group of patients. This assumption is not fully supported by the results of testosterone administration: this resulted in a rise of both plasma IGF-I and sTfR but these increases did not correlate.

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