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Estrogens Reduce Plasma Histidine-rich Glycoprotein (HRG) Levels in a Dose-dependent Way

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Summary

Plasma levels of histidine-rich glycoprotein (HRG) were investigated in three groups of women receiving a different dose of estrogens. First, the effect of low-dose estrogen was studied in a group of 83 postmenopausal women who were treated with 0.625 mg conjugated estrogens (CE). No significant change from baseline levels was found at the end of cycle 3 and cycle 13. Secondly, in 15 mothers and 23 daughters using oral contraceptives (OC) containing 30-50 μg ethinyl estradiol (EE) daily the mean HRG level was 14% and 24% lower than in a group of 144 mothers and 134 daughters not taking oral contraceptives, respectively ($p < 0.05$). Finally, in 11 excessively tall prepuberal girls who received 300 μg EE daily to reduce their final height the mean plasma HRG levels were decreased by 68% ($p < 0.005$). The effect of progestogens administered during low-dose and high-dose estrogen therapy appeared to be minor.

The results from these three studies indicate that estrogens reduce plasma HRG levels in a dose-dependent way.

Introduction

A dose-dependent, increased risk of thromboembolism has been reported to be associated with the estrogen component of oral contraceptives (1). In addition there have been some case reports of thrombosis during high-dose estrogen therapy, but the exact incidence is unknown (2,3). The reason for the increased risk is as yet unknown. One of the factors that may play a role is histidine-rich glycoprotein (HRG). Due to its interaction with haemostatic parameters like plasminogen (6), fibrin (7), and heparin (8), HRG is thought to act as a modulator of coagulation and fibrinolysis (4).

In a recent study individual plasma levels of HRG appeared to be very stable in time (16). During a period of 6 months only minor fluctuations were observed in HRG levels of 20 healthy volunteers and no evidence of seasonal fluctuations was found. The overall genetic influence on plasma HRG levels has been determined in a parent-twin study (15). 69% of the variance in HRG levels could be ascribed to genetic factors, the other 31% of the variance could be explained by individual environmental factors. So far only a few factors have been reported which lead to an alteration of the plasma HRG levels. In the majority of the cases a decrease is reported due to diseases like severe liver malfunction (17) and sepsis (18), or to immunosuppressive steroid therapy (19). Only in patients suffering from thrombosis (10,12) or mild (Child A) liver disease (17) is an increase seen. Furthermore HRG levels are negatively correlated with C-reactive protein (CRP) levels, indicating that HRG levels exhibit a negative response in the acute phase (20). In a few studies the marked effect of female sex hormones on the HRG level has been reported. A significant decrease of HRG levels (17-36%) has been found upon administration of oral contraceptives (21,22). Low levels of HRG have been observed during the third trimester of pregnancy with the lowest levels (40-50% of the normal level) at parturition (23). A small cyclic fluctuation of HRG levels has been observed during the menstrual cycle with the highest HRG levels during the early follicular phase, in which hormone levels are low (24).

To further evaluate the effect of female sex hormones on the HRG plasma level, we investigated the effect of various doses of estrogen in three different groups. The effect of low-dose estrogen was studied in a group of postmenopausal women receiving hormone replacement therapy. In a large sample of women from the general population the effect of an intermediate-dose estrogen was studied by comparing women using oral contraceptives with non-using women. The effect of high-dose estrogen was studied in prepuberal girls who received hormone therapy to reduce their final height.

Subjects and Methods

Low-dose Estrogen

Eighty-three postmenopausal women who had undergone hysterectomy were recruited by advertisement and articles in daily newspapers. Women between 50 and 65 years of age were included if serum Follicle Stimulating Hormone (FSH) was > 40 IU/l and serum 17-β-estradiol < 148 pM. Women were excluded if they had used oral estrogens and/or progestogens less than 3 months before the start of the study and if they had a positive history of thromboembolic disorders related to estrogen therapy. Two groups were formed by randomization. One group (CE) of 45 women (mean age ± s.d.: 54.9 ± 3.9) received 0.625 mg of conjugated estrogens (CE, Premarin®) continuously. The other group (CE + MDG) of 38 women (54.8 ± 4.0) received the same dosage of CE continuously plus 5 mg of medrogestone (MDG, Colprone®) the last 12 days of each cycle of 28 days (Premarin® and Colprone® are from Wyeth Laboratories, Hoofddorp, The Netherlands). Blood samples were taken in the morning after an overnight fast using vacutainer tubes containing EDTA as an anticoagulant. Plasma was prepared and stored at -70° C. Samples were thawed only once immediately before measurement. Baseline blood samples were taken within two weeks before the start of the therapy. During medication blood samples were drawn between the 22nd and 28th day of the 3rd and 13th cycle.

Intermediate-dose Estrogen

HRG levels of a large group of mothers and their daughters were measured in a parent-twin study previously described by Boomsma et al. (15). Mean ages (± s.d.) of mothers (n = 159) and daughters (n = 157) were 45 (± 5.4) and 17 (± 2.2), respectively. 9 mothers and 17 daughters used oral contraceptives with 30 μg ethinyl estradiol, 6 mothers and 6 daughters used oral contracep-

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tives with 50 μg ethinyl estradiol. 144 mothers and 134 daughters did not use oral contraceptives.

High-dose Estrogen

Eleven healthy prepuberal girls (mean age ± s.d.: 12.5 ± 1) were treated with high-dose estrogen to reduce their expected final height. They received 0.3 mg ethinyl estradiol daily and 5 mg of medroxyprogesteron-acetate every fourth week daily. Baseline blood samples were taken within one month before the start of the therapy. During medication blood samples were drawn once between 3-7 months after the start of therapy. 9 volumes blood were collected in vacutainer tubes containing 1 volume 3.2% sodium citrate as an anticoagulant. Plasma was prepared and stored in 0.2-0.5 ml aliquots at -70° C. Samples were thawed only once immediately before use.

HRG Measurement

Measurement of plasma HRG levels was performed by radial immunodiffusion (25) using 1% agarose plates with 7.5 mM veronal buffer (5,5-diethylbarbituric acid), pH 8.6 and 0.4% rabbit anti-HRG-antiserum. Rabbit polyclonal anti-HRG-antibodies were raised against purified human HRG kindly provided by Dr. N. Heimberger (Behringwerke, Germany). Plasma samples were diluted 1:4 in phosphate-buffered saline, pH 7.4, using an automated diluter (Hamilton Bonaduz A.G., Switzerland). Five μl of the diluted sample was placed in a well and allowed to diffuse at 4° C for 48 h. After washing with 0.9% sodium chloride for 48 h at 4° C, sharply delineated precipitation rings were observed. HRG concentrations were calculated from a standard dilution series of pooled plasma obtained from 26 healthy volunteers. Calibration lines were prepared both from EDTA and citrated pooled plasma to circumvent differences in measurements due to the use of EDTA or sodium citrate as anticoagulant in the different groups. HRG levels were expressed as a percentage of pooled plasma, taking citrated pooled plasma as 100%. The inter-assay coefficient of variation of the duplicate measurements was 10%.

Statistical Analysis

For all groups mean HRG levels and standard deviations (s.d.) or standard errors of the mean (s.e.m.) were calculated. ANOVA was used to test for differences in HRG level between the two treatment groups in the low-dose estrogen group and between users and non-users of OC in the intermediate-dose group. Differences in age and baseline HRG levels between groups were also tested with ANOVA. Repeated measures ANOVA was used to test for differences across time in the low-dose and high-dose estrogen group.

Table 1 Effect of low-dose estrogen treatment on plasma HRG levels in hysterectomized postmenopausal women

	Mean % HRG (s.e.m.)		
Group	Baseline	3 cycles	13 cycles
CE (n = 45)	113 (3.3)	111 (3.5)	112 (3.6)
Range	69-174	66-171	68-177
CE + MDG (n = 38)	106 (3.2)	106 (3.3)	104 (3.3)
Range	74-163	75-163	79-164

HRG levels and s.e.m. are expressed as a percentage of pooled plasma. CE: group receiving conjugated estrogen. CE + MDG: group receiving conjugated estrogen plus medrogestone.

Results

Effect of Low-dose Estrogen

HRG plasma levels were determined in two groups of hysterectomized postmenopausal women receiving hormone replacement therapy to evaluate the effect of low-dose estrogen. Mean HRG levels and ranges are shown in Table 1. Baseline HRG levels of the two treatment groups were not statistically significant. During treatment no significant change across time was observed both in the group receiving CE and in the group taking CE + MDG. The difference in response between the groups was also not statistically significant.

Effect of Intermediate-dose Estrogen

The effect of intermediate-dose estrogen was determined by comparing HRG levels of women using oral contraceptives to non-using wo-

Table 2 Mean HRG levels and range of mothers and daughters. Effect of oral contraceptives

	No OC	30 or 50 μg EE	30 μg EE	50 μg EE
Mothers (n)	144	15	9	6
Mean HRG (s.e.m.) %	102 (1.7)	88 (5.8) ($p < 0.05$)#	87 (7.3)	89 (8.7) NS*
Range	58-168	43-119	50-119	43-119
Daughters (n)	134	23	17	6
Mean HRG (s.e.m.) %	93 (1.8)	71 (4.6) ($p < 0.001$)#	75 (5.9)	62 (5.0) NS*
Range	49-168	36-118	36-118	46-76

P value of an unpaired t-test between the mean level of mothers or daughters using OC versus not using OC.
* P value of an unpaired t-test between users of OC containing 30 μg EE and 50 μg EE. NS = not significant.

Table 3 Mean HRG levels and range of 11 adolescent girls treated with high dose estrogen

	Baseline	During therapy
Mean HRG (s.e.m.) %	90 (6.2)	29 (3.9)
		(p = 0.005)*
Range	63-133	8-48

* P <0.05 in a repeated measures ANOVA.

men in a group of mothers and their daughters. Mean HRG levels are shown in Table 2. Mothers and daughters not using OC have significantly different mean HRG levels indicating an effect of age (see also Table 4). Differences between users of OC and non-users were therefore tested separately in mothers and daughters. Mothers (n = 15) using 30-50 μg EE have on average 14% lower HRG levels than non-using mothers (n = 144) ($p < 0.05$). The mean HRG level in daughters (n = 23) using 30-50 μg EE is 21% lower ($p < 0.001$) than in non-users (n = 134). Women taking OC can be divided in groups using OC containing 30 or 50 μg EE. Daughters using 50 μg EE tend to have a lower HRG level than daughters using 30 μg EE but this difference is not significant. In mothers there was no difference between users of 30 and 50 μg EE.

Effect of High-dose Estrogen

The effect of 300 μg EE on the HRG level has been evaluated in 11 prepuberal girls. The mean HRG level was lowered from 90% at baseline to 29% during therapy, which is an average decrease of 68% ($p < 0.005$) (Table 3). No effect was observed from differences in duration (3-7 months) of the therapy (data not shown).

Effect of Age on Baseline HRG Levels

The effect of age on HRG levels was studied using the baseline HRG levels of postmenopausal women, prepuberal girls and mothers and daughters not using OC. Mean ages and baseline HRG levels of the three groups are shown in Table 4. The mean age of the two treatment groups of postmenopausal women was not significantly different. Therefore the baseline levels of the two groups were combined.

Table 4 Baseline HRG levels and age

Group (n)	Age (s.e.m.)	Mean % HRG (s.e.m.)
Postmenopausal women (83)	55.0 (0.43)	110 (2.3)
Mothers (144)	46.0 (0.49)	102 (1.7)
Daughters (134)	16.2 (0.16)	93 (1.8)
Prepuberal girls (11)	12.5 (0.30)	90 (6.2)

Differences in age and mean HRG levels were tested using ANOVA. Except for the difference in HRG level of prepuberal girls versus daughters and prepuberal girls versus mothers, all differences both in age and HRG levels between the four groups were significant ($p < 0.05$).

ANOVA among the groups investigated revealed significant differences in age and mean HRG levels (Table 4). HRG levels tend to become higher as the age of the group studied increases: prepuberal girls (mean age = 12.5 yrs) and daughters (16.2 yrs) had about 10% lower HRG levels than mothers (46 yrs) ($p < 0.05$). Postmenopausal women (55 yrs) had on average 8% higher HRG levels than mothers ($p < 0.05$).

Discussion

The results of our study show a dose-dependent influence of estrogen on the HRG plasma level. During low-dose estrogen treatment in hormone replacement therapy, no effects on the HRG level were found. The use of oral contraceptives was associated with a decrease of the HRG level by 14-24% and the administration of high-dose estrogen leads to a lowering of the level by 68%. In clinical terms, a dosage of 625 μg of conjugated estrogens is comparable to 5 μg of ethinyl estradiol. Thus the low-dose estrogen group received the equivalent of 5 μg ethinyl estradiol while the oral contraceptive group received 30-50 μg and the high-dose estrogen group 300 μg.

A point that should be mentioned is that of the presence of different progestogens in the hormone preparations. In general progestogens are added during the estrogen treatment in a dosage just enough to prevent endometrial adenocarcinoma (26). In our study the dose of medrogestone received by the low-dose estrogen group was similar to that of medroxyprogesteron acetate in the high-dose estrogen group because both progestogens are derivatives of 17-hydroxy-progesterone and are thought to have comparable physiological effects (27). Since no effect of treatment on the HRG level was observed in the low-dose estrogen group, whereas a dramatic effect was observed in the high-dose estrogen group, it can be assumed that the progestogen component has a negligible influence on the HRG level. This is in support with the observations of Haukkamaa et al. (23), who reported that the subcutaneous administration of 60 μg/day of Progestin ST-1435 had no effect on the HRG level.

The groups in the present study differ markedly with respect to age and baseline HRG levels. HRG levels tend to increase with increasing age. A part of the age effect found in this study has previously been described by Boomsma et al. (15). They reported a generation effect in a parent-twin study. Both fathers and mothers have higher HRG levels than their sons and daughters, respectively.

In the present study, the increase in HRG seemed not to be linear with respect to time since an increase of 9% is observed in the 30 years between daughters and mothers whereas an increase of 8% is found in the 9 years between mothers and postmenopausal women. This may indicate an effect of the menopause. A related observation was done by Thompson et al. (28), who found that the increase of HRG was greater in women than in men after the age of 50. These results suggest a small age effect which is probably also present in men but in addition to this there is possibly also an effect of the menopause on the HRG level.

Whether the magnitude of the effect of estrogens is also age-dependent, remains unclear. Due to the design of the study it is not possible to draw any conclusions in this field.

From previous studies (21,22) and our own study it is obvious that the administration of exogenous estrogens reduces HRG levels. Several results indicate that there is also a relationship between endogenous oestradiol levels and HRG. Men have higher HRG levels than women (15), but this difference is reversed after the menopause (28) when oestradiol levels in women are decreased. HRG levels are low both during the last trimester of pregnancy (23) and day 12-16 of the menstrual cycle (24), when oestradiol levels are increased. However, no correlation

between serum HRG and total serum oestradiol was found in a cross-sectional study in women (23). Therefore, the possible relationship between endogenous oestradiol levels and plasma HRG levels needs to be studied in further detail.

Plasma HRG is synthesized by the liver (5) and although deviating HRG levels are observed mainly under circumstances with aberrant liver function (17), little is known about the regulation of the HRG level. Both conjugated estrogens and ethinyl estradiol have rather strong effects on several haemostatic parameters synthesized by the liver (22,29). It can therefore be speculated that estrogens reduce HRG levels via an alteration of the liver function. Whether this reduction is caused by reduced synthesis or increased clearance is as yet unclear.

In several studies, elevated levels of plasma HRG have been observed in groups of patients suffering from thrombosis (9-11) and, inherited elevation of HRG has been found in families with thrombosis (12-14). In addition, from in vitro experiments it has been suggested that elevated levels of HRG may inhibit plasminogen activation (6). However, the generation of plasmin is not modified in patients with high HRG levels (13) and up to now no evidence has been found for a causal relationship between high plasma HRG levels and thrombosis. The results from the present study show that a dose-dependent reduction of HRG plasma levels during treatment with female sex hormones is caused by the estrogen component. It seems therefore unlikely that plasma HRG levels are related to the increased risk of thromboembolism during estrogen treatment.

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