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Effects of Oral Contraceptives on Natriuretic Peptide Levels in Women with Hypothalamic Amenorrhea: A Pilot Study

Eleanor Lin, M.D.^a, Steven Grinspoon, M.D.^a, Thomas Wang, M.D.^b, and Karen K. Miller, M.D.^a

^aNeuroendocrine Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA

^bCardiology Division, Massachusetts General Hospital and Harvard Medical School, Boston, MA

Abstract

Natriuretic peptides, which are important regulators of salt handling and blood pressure, are 60 – 75% higher in healthy young women than in men, consistent with a gender dimorphism. In this randomized, placebo-controlled study in women with functional hypothalamic amenorrhea, we show that administration of oral contraceptives increases natriuretic peptide levels and that end-of-study free testosterone levels are inversely associated with NT-proBNP levels, consistent with the hypothesis that natriuretic peptide levels may be mediated by differences in gonadal steroid concentrations – estrogens or androgens.

Keywords

natriuretic peptides; oral contraceptives

The natriuretic peptide system, which counteracts the salt-handling effects of the renin-angiotensin system and may be an important regulator of arterial pressure, is characterized by significant gender dimorphism, with levels approximately 60% – 75% higher in healthy women compared to men (1, 2). However, the mechanisms responsible for the gender differences in the natriuretic peptide system have not been established, and few studies have examined the effects of gonadal steroids on this system (3–5). In this study, we investigated the effects of oral contraceptives compared to placebo administration on natriuretic peptide levels in a group of young, otherwise healthy, estrogen-deficient women with functional hypothalamic amenorrhea. We hypothesized that oral contraceptive administration would result in an increase in N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels and that this increase would correlate with decreased free testosterone levels.

Inclusion criteria were ages 18 to 40 years with: 1) no menses in the previous three months and $\leq$ two menses in the previous twelve months, 2) previous hypothalamic amenorrhea diagnosis and no spontaneous menses in the previous three months, or 3) previous hypothalamic amenorrhea diagnosis and total duration of hormonal therapy < six months

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Corresponding Author: Karen K. Miller, Neuroendocrine Unit, Bulfinch 457B, Massachusetts General Hospital, Boston, MA 02114, KKMILLER@Partners.org, Tel: 617-726-3870, Fax: 617-726-5072.

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during the previous year; body mass index (BMI) between 16 and 24 kg/m²; non-hormonal form of birth control; and fluency in English. All subjects were nonsmokers.

Exclusion criteria have been previously stated (6) and included any disorder for which gonadal steroid therapy was contraindicated; hypertension; recent hormonal therapy; LH/FSH $\geq$ 2.5 and LH $>$ 11 mIU/mL, and elevated testosterone level.

The protocol has been previously described elsewhere (6). Subjects participated in a multi-center double-blind, randomized, placebo-controlled study to determine the effects of oral contraceptives on bone markers in otherwise healthy women with functional hypothalamic amenorrhea. Investigators and subjects were blinded to the randomization assignment. Randomization was determined centrally with a 1:1 allocation ratio, variable block sizes and study centers as allocation strata. Color-matched placebo tablets were packaged centrally in identical sealed boxes with preprinted subject numbers. We report the effects of oral contraceptive (OCP) administration on natriuretic peptide levels from subjects who participated in the study from our center (n=15, with n=12 completers). The protocol was approved by the Partners Healthcare Institutional Review Board, and informed consent was obtained from all study subjects. Study visits took place from 1998–1999. Subjects were randomized to receive a triphasic, combination OCP containing norgestimate (NGM) and ethinyl estradiol (EE) (n=5) or placebo (n=7) for three consecutive 28-day cycles. The treatment group received NGM 180 μ g / EE 35 μ g on days 1–7, NGM 215 μ g / EE 35 μ g on days 8–14, NGM 250 μ g / EE 35 μ g on days 15–21, and inactive tablets on days 22–28. Fasting, early morning blood samples at the baseline and 3-month visits were assayed for NT-proBNP, testosterone, and sex-hormone binding globulin (SHBG). The 3-month blood draws were performed between days 15 and 21 of cycle 3 of the OCP/placebo tablets.

Commercially available assays were used for NT-proBNP (Elecsys proBNP Assay, Roche Diagnostics, Indianapolis, Indiana, total precision of 1.8–4.6%), SHBG (Architect SHBG Assay, Abbot Diagnostics, Chicago, Illinois, total precision of 5.65–9.54%), and testosterone (AxSYM Testosterone Assay, Abbot Diagnostics, Chicago, Illinois, total precision of 6.0–13.7%). The Architect second generation testosterone assay is accurate at testosterone concentrations relevant to female physiology (7). Free testosterone was calculated using the laws of mass action, which has been validated in women (8). Total body water (TBW) was assessed by bioelectric impedance analysis using Bioelectrical Analyzer model BIA 101 (RJL Systems, Michigan) using a standard protocol (9).

JMP statistical discovery (version 8.0.2; SAS Institute, Inc., Cary, NC) software was used for statistical analysis. After log transformation, means between groups were compared using ANOVA, and change in NT-proBNP within groups was analyzed by paired t-tests. For linear regressions, data were fit to a logarithmic trendline, and Pearson coefficients are reported.

At baseline, the OCP and placebo groups were of comparable age (25 $\pm$ 4 [SD] vs. 27 $\pm$ 7 years, range 19–37 years), blood pressure (SBP 103 $\pm$ 8 vs. 110 $\pm$ 17, DBP 65 $\pm$ 15 vs. 67 $\pm$ 14, MAP 78 $\pm$ 12 vs. 81 $\pm$ 15 mmHg), BMI (19.4 $\pm$ 1.2 vs. 19.8 $\pm$ 1.9 kg/m²), TBW (30 $\pm$ 4 vs. 30 $\pm$ 3 L), NT-proBNP levels (38.5 $\pm$ 26.9 vs. 30.7 $\pm$ 14.7 pg/mL), LH (3.2 $\pm$ 2.5 vs. 7.2 $\pm$ 4.5 mIU/mL) and FSH (6.0 $\pm$ 3.0 vs. 6.2 $\pm$ 2.0 mIU/mL), the latter two consistent with a diagnosis of hypothalamic amenorrhea (10, 11). Mean time since last menstrual period was comparable between the groups (38 $\pm$ 49 vs. 21 $\pm$ 21 months). There was a trend toward a difference in baseline free testosterone levels (3.7 $\pm$ 1.4 vs. 6.8 $\pm$ 4.3 pg/mL, $p=0.09$).

Mean NT-proBNP levels increased significantly in the OCP group compared with placebo (Fig 1A; $p=0.0002$). Mean NT-proBNP levels increased from 38.5 $\pm$ 12.0 [SEM] pg/mL to 72.1 $\pm$ 23.1 pg/mL in the OCP treatment group ($p=0.05$), whereas there was no change in the

placebo group (mean NT-proBNP 30.7 ± 5.6 at baseline and 27.7 ± 4.7 pg/mL at 3 months, $p=0.19$). All subjects in the OCP group experienced numerical increases in their NT-proBNP levels over the 3-month period (20–95 pg/mL). The baseline NT-proBNP levels were comparable to median levels in healthy blood donors, whereas the post-OCP treatment levels approached the 90th percentile (12). There was no change in BMI ($p=0.84$), TBW ($p=0.45$), percent TBW ($p=0.77$), or blood pressure (SBP $p=0.44$, DBP $p=0.86$, MAP $p=0.67$) from baseline to three months. None of the subjects developed edema during the study.

At three months, NT-proBNP levels inversely correlated with free testosterone (Fig 1B; $R=-0.64$, $p=0.024$). At baseline, it was not statistically significant ($R=0.16$, $p=0.62$), and change in NT-proBNP levels versus change in free testosterone was not correlated ($R=-0.46$, $p=0.21$).

Our data demonstrate that OCP administration increases natriuretic peptide levels compared to placebo. Our data also demonstrated a significant association between NT-proBNP and free testosterone levels after OCP administration. Therefore, the effects observed may be due to a decrease in androgen, rather than the increase in estrogen, levels. Further studies are warranted to elucidate the effects of androgens and estrogens on the natriuretic peptide system and to determine whether gonadal steroid effects are responsible for the sex differences in natriuretic peptide levels and/or contribute to the dichotomous sex-specific risk for the development of hypertension. In addition, OCP use is associated with the development of hypertension in a minority of women, and the pathophysiologic basis for this complication is unknown. Further studies are warranted to determine whether the increase in natriuretic peptide levels induced by OCPs serves a protective role against the development of hypertension in a larger number of women.

The role of natriuretic peptides in healthy individuals is an emerging field. Natriuretic peptides increase natriuresis and diuresis, counteract the renin-angiotensin system, and decrease blood pressure. As the incidence of cardiovascular disease and hypertension is lower in premenopausal women than in age-matched men, the question has been raised as to whether the dichotomous natriuretic peptide levels may play a role (13, 14). Genetic variation at the proBNP gene locus has been linked to natriuretic peptide levels in a genome-wide association (15), and alleles associated with higher BNP levels were also associated with lower blood pressure (16), suggesting a putative role for natriuretic peptides as determinants of blood pressure in healthy individuals.

In healthy women, natriuretic peptide levels are higher compared to men, but the basis for this difference is not understood (1, 2, 12, 17). Animal studies have suggested an important role for gonadal steroid regulation of the natriuretic peptide system (18–21). Human data also suggest a role for gonadal steroids in the determination of natriuretic peptide levels. In an uncontrolled study of 22 postmenopausal women on estrogen/progestin therapy for three months, ANP and BNP levels significantly increased (progestins and androgens not measured) (4). A cross-sectional study of 682 pre-menopausal women showed that total testosterone, free androgen index, and calculated free testosterone levels were inversely associated with BNP and NT-proBNP levels, raising the possibility that androgens are important determinants of natriuretic peptide levels, but menopausal status and FSH levels (estradiol levels were not measured) were not associated with NP levels (3). This study points towards androgens rather than estrogens/progestins as potential regulators of the natriuretic peptide system. We report a strong inverse association between free testosterone and NP levels after oral contraceptive administration. This suggests that the effects of oral contraceptive administration on NP levels may be mediated by the decrease in free androgen levels. However, a combination oral contraceptive was given, so it was not possible to

isolate the effects of the estrogen and progestin components, and changes in estrogens and androgens are strongly correlated with each other. Therefore, we cannot rule out a direct effect of estrogen and/or progestin administration on the NP system.

In conclusion, in women with hypothalamic amenorrhea, OCPs for three months increased NT-proBNP levels compared with placebo. After the intervention, NT-proBNP levels were strongly inversely associated with free testosterone levels, suggesting an androgen effect, but we cannot rule out effects of other gonadal steroids. Further studies are warranted to investigate whether androgens, estrogens and/or progestins are responsible for the differences in natriuretic peptide levels observed between the sexes.

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Figure 1.

Panel A. Change in NT-pro BNP after three months of treatment with OCP or placebo are shown. The OCP group had a statistically significant increase in NT-pro BNP levels compared with placebo ($p=0.0002$). **Panel B.** Correlation of NT-pro BNP with calculated free testosterone levels in the OCP group. The logarithmic curve has an $R=-0.64$ and $p=0.024$.