British Journal of Obstetrics and Gynaecology
May 1980. Vol. **87.** pp 416–421

EFFECTS ON SEX HORMONE BINDING GLOBULIN OF DIFFERENT ORAL CONTRACEPTIVES CONTAINING NORETHISTERONE AND LYNESTRENOL

BY

VIVECA ODLIND

E. WEINER

A. VICTOR

AND

E. D. B. JOHANSSON

Department of Obstetrics and Gynaecology
University Hospital, Uppsala, Sweden

Summary

Six combined oral contraceptive drugs containing ethinyloestradiol or mestranol and norethisterone or lynestrenol were studied throughout six 21-day cycles in healthy female volunteers. There was always one menstrual cycle without treatment between every treatment cycle. Plasma levels of norethisterone were determined throughout treatment by use of a specific radioimmunoassay. Sex hormone binding globulin (SHBG) was measured by an ammonium sulphate precipitation method at the beginning and at the end of each treatment cycle. The results indicated an accumulation of norethisterone in plasma when 1 to 3 mg of norethisterone or lynestrenol was given. There was no obvious accumulation during treatment with 0·5 mg norethisterone. SHBG increased during treatment with all combinations studied. However, this increase was most pronounced with the 50 μg ethinyloestradiol/1 mg lynestrenol, 50 μg mestranol/1 mg norethisterone and 35 μg ethinyloestradiol/0·5 mg norethisterone combinations. There was no statistically significant increase in SHBG with 50 μg ethinyloestradiol/2·5 mg lynestrenol or 50 μg ethinyloestradiol/3 mg norethisterone acetate combinations. The results indicated that the induction of SHBG by the synthetic oestrogens was antagonized by the progestogens in a dose-dependent manner. The effect on SHBG by a combined preparation could be one assessment of the oestrogenicity or androgenicity of the preparation.

NORETHISTERONE, lynestrenol and levonorgestrel are the commonly used progestogens and ethinyloestradiol or, less frequently, mestranol the oestrogens in oral contraceptive pills. Synthetic oestrogens induce production of sex hormone binding globulin (SHBG; Anderson, 1976). This effect is antagonized by androgens and by the progestogens derived from 19-nortestosterone (Anderson, 1976). As suggested by van Kammen *et al* (1974) the effects of the combined preparations on SHBG could therefore give an estimate of the oestrogenic potency of the preparation.

Levonorgestrel has previously been shown to

bind strongly to SHBG, whereas norethisterone has a less pronounced affinity (Victor *et al*, 1976). Lynestrenol differs chemically from norethisterone only in the absence of an oxo group in position 3. Lynestrenol administration produces plasma concentrations of norethisterone in the same range as found during norethisterone treatment indicating a conversion of lynestrenol to norethisterone (Odlind *et al*, 1979).

Previous studies on levonorgestrel and norethisterone have shown an accumulation of the drugs, reflected by increasing plasma concentrations of these steroids during long-term treatment (Weiner *et al*, 1976; Braselton *et al*, 1979). This study was performed in order to find whether or not such a build-up is due to a simultaneous increase of SHBG during cyclical treatment with oral contraceptives containing norethisterone or lynestrenol in combination with either ethinyloestradiol or mestranol.

Methods

Five healthy women, 19 to 31 years old, were enrolled in the study. The preparations used in the study are shown in Table I. Two left the study for personal reasons after having taken four of the preparations. Two new volunteers, 34 and 38 years old, respectively, were enrolled for the study of Lyndiolett and Anovlar. They had all a previous history of regular menstruations and were on no medication.

The subjects completed one cycle on each preparation with one untreated menstrual cycle between every treatment cycle. The first tablet was always taken on day 5 and then at 24 hours intervals for a total of 21 days.

Blood samples were collected before the first tablet and then 12 hours after each tablet was taken. Blood samples were drawn from an antecubital vein into heparinized glass tubes. Plasma was withdrawn after centrifugation and kept below −17°C until analysed.

Norethisterone was determined by a radioimmunoassay (Odlind *et al*, 1979). SHBG was measured as dihydrotestosterone binding capacity of plasma in nmol/l as described by Rosner (1972). All samples were analysed for norethisterone. SHBG was determined in the samples taken just before treatment and the samples from the three first and four last days of treatment. Student's t-test was used for statistical calculations.

Table I

Oral contraceptive combinations used in the study

Preparation	Progestogen dose	Oestrogen dose* (μg)
Anovlar	3 mg norethisterone acetate	50
Lyndiol	2·5 mg lynestrenol	50
Brevicon	0·5 mg norethisterone	35
Norminest	0·5 mg norethisterone	60 every other day
Conlumin	1 mg norethisterone	50 mestranol
Lyndiolett	1 mg lynestrenol	50

* Ethinyl oestradiol unless otherwise stated

Results

The plasma levels of norethisterone 12 hours after ingestion of the different preparations are seen in Figure 1 as mean of five subjects during three consecutive days. The individual plasma levels of norethisterone varied greatly (Table II). The mean of four SHBG determinations in each

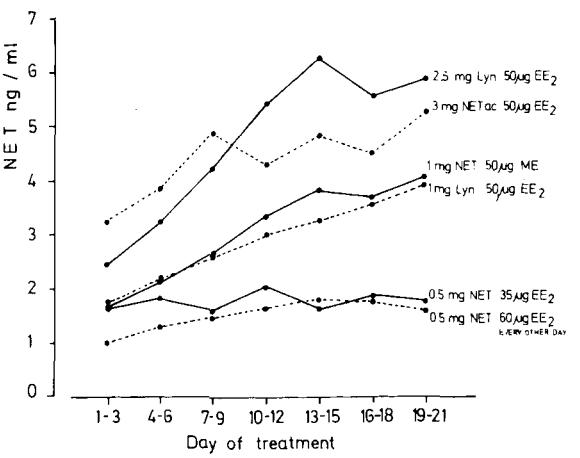

Fig. 1

Plasma levels of norethisterone (ng/ml) during treatment with six different oral contraceptive combinations. Each point represents the mean level in five women during three consecutive days of treatment. ME = mestranol; EE_2 = ethinyloestradiol; NET = norethisterone; Lyn = lynestrenol.

subject at the beginning and the mean of four at the end of each treatment cycle are seen in Figure 2.

0·5 mg norethisterone

During treatment with Brevicon and Norminest, which both contain 0·5 mg norethisterone, mean plasma levels of norethisterone ranged between 1·0 ng/ml and 2·0 ng/ml. There was no statistically significant difference between the norethisterone levels found after treatment with either of these preparations except in the very first samples when the mean norethisterone levels were significantly lower after Norminest ($p < 0·01$).

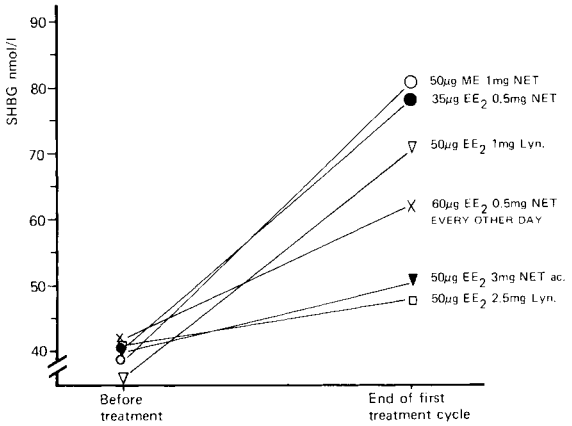

FIG. 2

Plasma SHBG levels (nmol/l) before and at the end of a treatment cycle with six different oral contraceptive combinations. Each point represents the mean of 3 to 4 determinations in five women. ME = mestranol; EE_2 = ethinyloestradiol; NET = norethisterone; Lyn = lynestrenol.

1 mg norethisterone and 1 mg lynestrenol

When Conlumin was given, mean plasma levels of norethisterone were initially 1·65 ng/ml, then gradually increased during the first 14 days of treatment. The mean norethisterone levels varied between 3·5 ng/ml and 4·0 ng/ml for the rest of the cycle. During Lyndiolett treatment, mean plasma norethisterone levels increased throughout treatment from 1·75 ng/ml to around 4 ng/ml. The mean norethisterone levels were never statistically different from those found during treatment with Conlumin. The mean plasma levels of norethisterone during Conlumin and Lyndiolett treatment were statistically higher from days 7 to 9 onwards than the mean norethisterone levels during treatment with the 0·5 mg norethisterone preparations (p 0·001–0·005).

2·5 mg lynestrenol and 3·0 mg norethisterone acetate

During treatment with Lyndiol mean plasma norethisterone levels increased rapidly from

TABLE II

Plasma levels of norethisterone (in 5 patients) during treatment with six different oral contraceptive combinations

	Plasma levels of norethisterone (ng/ml) Mean ± SE†						
	Days of treatment						
Preparation*	1–3	4–6	7–9	10–12	13–15	16–18	19–21 (22)
Anovlar	3·23±1·03	3·77±0·54	4·87±0·65	4·28±0·73	4·82±0·85	4·52±0·74	5·28±1·16
Conlumin	1·65±0·32	2·11±0·31	2·62±0·36	3·33±0·60	3·79±0·56	3·69±0·58	4·06±0·65
Brevicon	1·62±0·32	1·79±0·45	1·59±0·29	2·03±0·40	1·62±0·31	1·88±0·33	1·76±0·26
Norminest	0·99±0·22	1·29±0·23	1·47±0·22	1·63±0·18	1·79±0·14	1·76±0·21	1·59±0·14
Lyndiol	2·44±0·42	3·24±0·67	4·23±1·13	5·41±1·47	6·27±2·25	5·55±1·62	5·90±1·76
Lyndiolett	1·75±0·64	2·15±0·86	2·59±0·80	2·97±0·68	3·24±0·65	3·56±0·88	3·92±0·77

* For constituents of preparation see Table I.
† Mean of three days in five women ±SE.

2·44 ng/ml to around 6·0 ng/ml during the first 14 days. Then the mean norethisterone levels stabilized around 5·5 ng/ml for the rest of the cycle. Mean plasma norethisterone levels during Anovlar treatment were initially around 3·0 ng/ml, then increased slowly to almost 5 ng/ml during the first nine days of treatment. Another slight increase of mean norethisterone levels was seen at the end of treatment. The mean norethisterone levels found during Anovlar treatment did not differ statistically from those found during Lyndiol treatment. The mean norethisterone levels found during Anovlar and Lyndiol treatment were statistically higher than those found during Conlumin and Lyndiolett treatment (p 0·01–0·05).

SHBG

SHBG varied within normal levels in the beginning of each treatment cycle. At the end of the Brevicon cycle the mean increase of SHBG was 94 per cent ($p < 0·001$). Only half of that increase was found at the end of the Norminest treatment period, 45 per cent ($p < 0·01$). During the Conlumin treatment cycle the mean increase of SHBG was 105 per cent ($p < 0·001$) and SHBG increased 100 per cent during Lyndiolett treatment ($p < 0·001$). During treatment with Anovlar the mean SHBG increased 30 per cent (not significant). Lyndiol treatment resulted in the least increase of SHBG, namely 17 per cent.

A positive correlation between plasma norethisterone concentrations and SHBG was found during Conlumin and Lyndiolett treatment using a linear regression analysis ($r = 0·88$, $p < 0·001$). No correlation was found between norethisterone levels and SHBG for any of the other preparations.

Discussion

Ethinyloestradiol is known to induce a pronounced rise in SHBG. Briggs (1975) showed a more than 200 per cent rise in SHBG in women who had been given 30 μg and 50 μg ethinyloestradiol. This increase was unaffected by the addition of 1 mg lynestrenol to 50 μg ethinyloestradiol. Van Kammen *et al* (1975), however, could demonstrate that, in combination with 2·5 mg lynestrenol, 50 μg ethinyloestradiol did not produce a significant rise of SHBG. Neither was there an increase of SHBG in women treated with 50 μg ethinyloestradiol together with 0·25 mg levonorgestrel.

This study confirms the results of van Kammen *et al* (1975) showing that the combination of 2·5 mg lynestrenol and 50μg ethinyloestradiol (Lyndiol) caused no statistically significant rise in SHBG. However, the combinations of 1 mg norethisterone or lynestrenol with 50 μg mestranol or ethinyloestradiol (Conlumin, Lyndiolett) caused a significant rise in SHBG. Our results, together with those of Briggs (1975) and van Kammen *et al* (1975), indicate a dose-dependency between the progestogen and the effect on SHBG rather than a substance-related effect. The effect on SHBG is equally dependent of the dose of ethinyloestradiol and the dose interval of ethinyloestradiol. This is demonstrated by the less pronounced rise in SHBG found during Norminest treatment when compared to Brevicon (0·5 mg norethisterone, 35 μg ethinyloestradiol). In Norminest, 0·5 mg norethisterone was administered daily and the additional 60 μg ethinyloestradiol only every other day.

The mean plasma levels of norethisterone increased during the initial 12 to 14 days of treatment with Conlumin (1 mg norethisterone, 50 μg mestranol) before they reached a steady state. When Lyndiolett (1 mg lynestrenol, 50 μg ethinyloestradiol) was given the mean norethisterone concentration increased throughout treatment. The half life of norethisterone after a single oral dose of norethisterone or lynestrenol has previously been calculated to be around eight hours at 8 to 24 hours after administration (Odlind *et al*, 1979). It is thus unlikely that the accumulation found was due to a long half-life of the drug. The time interval before steady state was reached could be due to the simultaneous increase in SHBG, gradually allowing a larger portion of the steroid to be protein bound. Vermeulen *et al* (1969) showed that the metabolic clearance rate of testosterone increased with decreasing SHBG capacity. In parallel, it is probable that the metabolic clearance rate of the progestogen would decrease with increasing SHBG, due to a lowering of the ratio between free and protein-bound steroid. This is supported by the correlation found between plasma SHBG and norethisterone levels during Conlumin and

Lyndiolett treatment. Such a correlation between SHBG and the progestogen has previously been reported for levonorgestrel (Victor *et al*, 1977). Back *et al* (1978), studying a combination of norethisterone and ethinyloestradiol, also found a positive correlation between SHBG and plasma norethisterone levels. However, the lack of such a correlation for the other dosages tested indicate that the affinity of norethisterone to SHBG is less pronounced than that of levonorgestrel. The correlation found for the 1 mg dose of norethisterone or lynestrenol and 50 μg mestranol or ethinyloestradiol may only be coincidental and does not necessarily indicate a strong binding of the progestogen to SHBG.

During Lyndiol (2·5 mg lynestrenol, 50 μg ethinyloestradiol) treatment, plasma levels of norethisterone increased gradually to reach a plateau at the end of the second treatment week. The mean plasma norethisterone level was, however, only 1·6 times higher than during Lyndiolett treatment although the dose was 2·5 times higher. The increase was not accompanied by a simultaneous increase in SHBG. This build-up could only partly be explained by the longer terminal half life of norethisterone found after a single dose lynestrenol ($t_{\frac{1}{2}}$ 24 to 72 hours = 16 hours; Odlind *et al*, 1979), and could indicate an accumulation of the steroid at this dosage.

SHBG increased almost as much during Brevicon (0·5 norethisterone, 35 μg ethinyloestradiol) treatment as during Conlumin (1 mg norethisterone, 50 μg mestranol) treatment, although mean plasma levels of norethisterone did not increase during Brevicon administration. This may suggest that a high proportion of the progestogen is protein bound when given in a dose too small to satisfy all the binding sites of the carrier protein, and little is biologically available. This could be an additional explanation for bleeding problems that are common during treatment with low dose contraceptive pills.

This study has shown great individual difference in plasma norethisterone concentrations when the drugs were given under non-standardized, clinical conditions. The results indicate that the induction of SHBG by the synthetic oestrogens is antagonized by the progestogen and that this antagonism is dose dependent. The effect of the combined preparation upon SHBG could be an assessment of the oestrogenicity or androgenicity of a preparation, which allows more individualized and rational treatment with oral contraceptive pills. Women suffering from 'androgenic' symptoms such as acne vulgaris or hirsutism could benefit from a more oestrogenic preparation. Such a preparation would not only reduce the ovarian testosterone production, but also reduce the biological availability of testosterone by increasing SHBG levels. Whether the effects on SHBG, exerted by the oestrogens and progestogens, influence the amount of free steroids is a matter for further study.

Acknowledgements

This study was supported by the Ford Foundation, the Swedish Medical Research Council (Grant No. 03495), the International Committee for Contraception Research of the Population Council and Astra Syntex AB, Södertälje, Sweden.

References

Anderson, D. C. (1976): The role of sex hormone binding globulin in health and disease. In: *The Endocrine Function of the Human Ovary*. Edited by V. H. T. James, M. Serio, and G. Giusti. Academic Press, London.

Back, D. J., Breckenridge, A. M., Crawford, F. E., McIver, M., Orme, M. L'E., Park, B. K., Rowe, P. H., and Smith, E. (1978): Kinetics of norethindrone in women I. Radioimmunoassay and concentrations during multiple dosing. *Clin Pharmacol Ther*, **24**, 439–47.

Braselton, W. E., Lin, T. J., Ellegood, J. O., Mills, T. M., and Mahesh, V. B. (1979): Accumulation of norethindrone and individual metabolites in human plasma during short- och long-term administration of a contraceptive dosage. *Am J Obstet Gynecol*, **133**, 154–60.

Briggs, M. H. (1975): Hormonal contraceptives and plasma sex hormone binding globulin. *Contraception*, **12**, 149–53.

Odlind, V., Weiner, E., Victor, A., and Johansson, E. D. B. (1979): Plasma levels of norethindrone after single oral dose administration of norethindrone and lynestrenol. *Clin Endocrinol (Oxf)*, **10**, 29–38.

Rosner, W. A. (1972): A simplified method for the quantitative determination of testosterone-estradiol binding globulin activity in human plasma. *J Clin Endocrinol Metab*, **34**, 983–8.

Van Kammen, E., Thijssen, J. H. H., Rademaker, B., and Schwarz, F. (1975): The influence of hormonal contraceptives on sex hormone binding globulin (SHBG) capacity. *Contraception*, **11,** 53–9.

Weiner, E., Victor, A., and Johansson, E. D. B. (1976): Plasma levels of d-norgestrel after oral administration. *Contraception*, **14,** 563–70.

Vermeulen, A., Verdonck, L., van der Straeten, M., and Orie, N. (1969): Capacity of the testosterone-binding globulin in human plasma and influence of specific binding of testosterone on its metabolic clearance rate. *J Clin Endocrinol*, **29,** 1470–80.

Victor, A., Weiner, E., and Johansson, E. D. B. (1976): Sex hormone binding globulin: The carrier protein for d-norgestrel. *J Clin Endocrinol Metab*, **43,** 244–7.

Victor, A., Weiner, E., and Johansson, E. D. B. (1977): Relation between sex hormone binding globulin and d-norgestrel levels in plasma. *Acta Endocrinol*, **86,** 430–6.