SERUM LEVELS AND PHARMACOKINETICS OF NORETHISTERONE AFTER INGESTION OF LYNESTRENOL: ITS RELATION TO DOSE AND STAGE OF THE MENSTRUAL CYCLE

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Abstract

The peak concentration, peak time, the area under the serum concentration time curve (AUC) and half-life of serum norethisterone (NET) after a single application of lynestrenol (LYN) to female volunteers demonstrated that 0.7 mg NET is bioequivalent to 1 mg LYN which is rapidly converted to NET. There was a decrease of the peak values and an increase of half-life of NET during the periovulatory and luteal phase which was, however, not significant due to the great individual differences. The shift of the peak time to longer intervals and the increase of half-life of NET after ingestion of higher LYN doses indicate a certain limitation of the metabolic capacity of the liver. One of the volunteers who complained of nausea and vertigo after the administration of 5 mg LYN, showed the highest serum values of NET. The large interindividual variations of the serum levels of synthetic steroids demonstrate a possible risk of contraceptive safety in women with low steroid levels and possibly a coherence between extremely high serum levels of synthetic steroids and side effects.

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CONTRACEPTION

Introduction

The 4-en-3-one group is believed to be one of the most important structural characteristics of the progesterone molecule with respect to its progestogenic effects. When highly potent progestogens, e.g. lynestrenol or ethynodiol diacetate, were synthesized, lacking the keto-group at position 3, this concept seemed to be put in question. Subsequent investigations upon the metabolism of lynestrenol and ethyndiol diacetate (reviewed by Fotherby (1)), however, suggested that the metabolic pathway of both steroids involves the conversion of the 4-en-3-one-steroid norethisterone, a commonly used progestogen. Moreover, it could be demonstrated that the weak binding affinities of lynestrenol and ethynodiol diacetate to the uterine progesterone receptor (2, 3, 4) do not correspond at all to their marked hormonal potency.

It was, therefore, concluded that the progestogenic effect of both lynestrenol and ethynodiol diacetate is dependent on a preceding hepatic transformation into norethisterone which then is the active compound.

As a consequence, the contraceptive safety of preparations containing lynestrenol is based on a sufficient metabolic capacity of the liver which can be influenced by many drugs and various steroids, particularly estrogens.

In view of that we investigated the time course of the concentration of norethisterone in the serum of women after oral application of single tablets containing various dosages of lynestrenol with regard to a possible influence of the stages of the menstrual cycle.

Material and Methods

Six healthy female volunteers, ages between 21 and 28 years, having regular ovulatory cycles participated in this study. All women had not used hormonal contraceptives within one year before the beginning of the study.

On Day 2 or Day 5 of the cycle (early follicular phase), every woman ingested 0.7 mg NET (2 tablets of Conceplan® micro, Grünenthal) or 1 mg LYN (2 tablets of Exlutona®, Organon). One Day 15 (periovulatory phase) and on Day 21 (luteal phase), they ingested 1 mg LYN (2 tablets of Exlutona®, Oreganon). On Day 2 of the following cycle, each woman ingested 2 mg of LYN (4 tablets of Exlutona®, Organon) and, on Day 2 of the third cycle, 5 mg of LYN (1 tablet of Orgametril®, Organon). The tablets were taken with 100 ml of water before breakfast. No other drugs were applied during the experiment.

Blood samples (10 ml) were collected immediately before and 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 12 hours after the ingestion of the tablets from an antecubital vein. After centrifugation the serum samples were frozen and stored at -20°C until assayed.

The serum level of NET was determined by means of a radioimmunoassay kit kindly provided by Dipl. Ing. Nieuweboer (Schering, Berlin). 15,16-^{3}H-norethisterone with a specific activity of 57 μCi/nmole was used as tracer. The antiserum was lyophilized anti-norethisterone-11α-succinoyloxy-BSA (rabbit B3) diluted 1:10,000 (binding capacity 35%). The standard curve was in the range of 0.0685 to 7.5 ng/ml. After extraction of 0.2 ml serum with 2 ml diethylether and evaporation of the solvent, the assay was carried out in triplicate (incubation 16 hours at 4°C); buffer: Na-K-phosphate (M/15), pH 7.0, NaCl (M/15), NaN_3 (0.1% w/v), BSA (0.5% w/v). Free steroid was separated by dextrane-coated charcoal.

Cross-reactions: Lynestrenol < 0.08%, progesterone <0.08%, estradiol-17β < 0.08%, cortisol < 0.08%, 17α-ethinyl estradiol <0.08%, 17α-ethinyl-5α-estrane-3α, 17β-diol <0.08%, 17α-ethinyl-5β-estrane-3α, 17β-diol < 0.08%, testosterone 0.9%, 17α-ethinyl-17β-hydroxy-5β-estrane 2.2%, norethisterone enanthate 2.4%, 17α-ethinyl-17β-hydroxy-5α-estrane 16.6%.

Serum concentrations of estradiol and progesterone were determined by means of commercial RIA kits in serum samples taken immediately before the ingestion of the tablets.

The half-life was determined using the formula

$$t_{1/2} = 0.693 \cdot k^{-1},$$

where k is the slope of the regression line calculated for the natural logarithms of the serum NET concentrations following the peak value during the first 6 or 8 hours (at least 3 values).

The areas under the serum concentration time curves (AUC) were calculated by the trapezoidal rule. The correlation between doses and AUC_{0-12} was calculated by means of regression between log dose and AUC_{0-12}.

Results

The serum level of NET reached a maximum between 1 to 2 hours after the ingestion of 0.7 mg NET during the early follicular phase (Fig. 1). There were, however, conspicuous individual differences in the peak values varying from 6.6 to 34.2 ng/ml. Contrary to that, the subsequent decline in the serum level did not differ much.

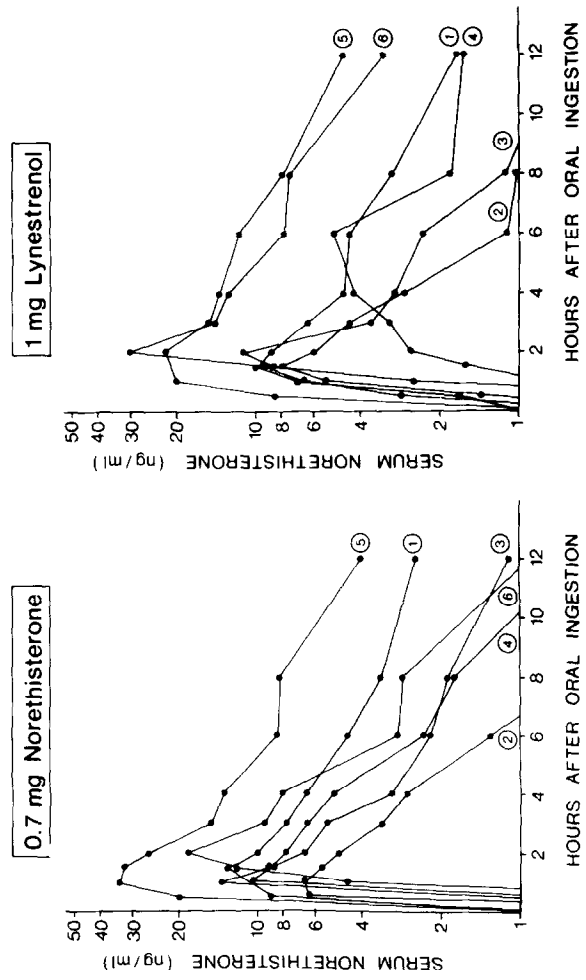

Fig. 1. Serum NET concentrations in women following oral administration of 0.7 mg NET or 1 mg LYN.

When 1 mg LYN was ingested during the early follicular phase, there was a rapid conversion to NET. The mean concentration of NET corresponded at all times to that obtained after the application of 0.7 mg NET. No significant differences could be found in the average half-life, the AUC_{0-12}, and the peak concentration of serum NET when either compound had been applied (Table I). Nevertheless, the interindividual variation was greater after the ingestion of 1 mg LYN as compared to 0.7 mg NET. In one of the volunteers, the metabolization of LYN occurred rather slowly, as the peak value after 6 hours did not exceed 5 ng/ml. The average time interval between ingestion and the peak concentration of NET tended to be greater when 1 mg LYN was used (Table I); the difference was, however, not significant.

When 1 mg LYN was taken at various stages of the menstrual cycle, there appeared to be a trend towards lower peak values of NET(Fig. 2) and an increase in half-life (Table I) during the periovulatory and even more so during the luteal phase. The differences were not significant due to great individual variations (Table II).

The application of higher doses of LYN resulted in a shift of the NET peak in that the maxima were reached later (Fig. 3). At the dose of 5 mg, the maximum occurred 3 to 4 hours after the application. The NET concentration in serum reached values ranging from 9.6 to 32 ng/ml after the ingestion of 2 mg LYN, and from 14.3 to 55 ng/ml at the dose of 5 mg. The half-life of NET in serum seemed to increase with the dose. There was, however, no significance between the average values (Table I). The regression between the AUC_{0-12} and the logarithms of the doses was significant ($p < 0.01$). The interindividual variation after the administration of 5 mg LYN was not as pronounced as compared to that of 1 mg or 2 mg. Serum NET was found to be in a range from 4.9 to 12.9 ng/ml after 12 hours.

It was noteworthy that a volunteer who complained of nausea and vertigo after the ingestion of 5 mg LYN, showed the highest serum NET concentration (peak value of 55.3 ng/ml, and 12.9 ng/ml after 12 hours).

Discussion

There is little doubt that LYN exerts its progestogenic effects after being converted metabolically (5, 6). As there was nearly no cross-reaction of LYN with the NET antibody in the RIA system used, the rapid rise of serum NET after ingestion of LYN reflects in fact its immediate conversion into the hormonally active compound which in all probability takes place in the liver (7).

Table I : *Pharmacokinetic parameters* (mean ± S.D.) of serum NET in 6 women after single ingestion of 0.7 mg NET or various doses of LYN

Dose	AUC_{0-12} (ng · h/ml)	peak concentration (ng/ml)	time to peak (h)	half-life (h)
0.7 mg NET (follicular phase)	63.8 ± 43.9	16.3 ± 9.7	1.3 ± 0.4	2.5 ± 1.0
1 mg LYN (follicular phase)	64.1 ± 46.5	14.6 ± 9.9	2.5 ± 1.7	2.5 ± 1.2
1 mg LYN (periovulatory phase)	53.9 ± 31.9	12.3 ± 6.5	1.8 ± 0.6	3.2 ± 1.2
1 mg LYN (luteal phase)	60.3 ± 47.6	11.3 ± 8.7	2.3 ± 1.0	3.9 ± 3.2
2 mg LYN (follicular phase)	114.4 ± 35.8	19.5 ± 8.1	3.2 ± 1.7	4.0 ± 1.4
5 mg LYN (follicular phase)	164.2 ± 83.8	26.3 ± 17.5	3.6 ± 0.6	4.3 ± 1.6

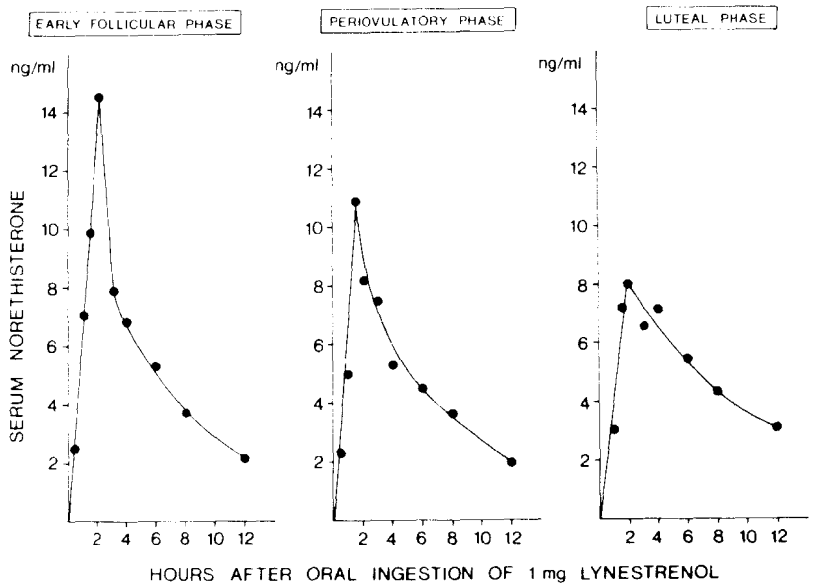

Fig.2. Average serum NET concentrations in women following oral administration of 1 mg LYN during various stages of menstrual cycle.

TableII: Time-course of serum concentrations of NET after oral administration of 1 mg LYN in relation to the stage of menstrual cycle (mean ± S.D.)

	Early follicular phase	Periovulatory phase	Luteal phase
Serum estradiol (pg/ml)	37.3 ± 27.0	256.2 ± 251.6	121.3 ± 56.9
Serum progesterone (ng/ml)		0.5 ± 0.5	7.8 ± 5.2
Serum NET (ng/ml)			
0 h	0.3 ± 0.4	0.2 ± 0.3	0.0 ± 0.0
0.5 h	2.5 ± 3.1	2.3 ± 2.8	0.9 ± 0.3
1 h	7.1 ± 6.8	4.9 ± 2.7	5.0 ± 5.5
1.5 h	9.9 ± 6.5	10.9 ± 6.7	7.2 ± 9.3
2 h	14.5 ± 12.4	8.2 ± 4.4	8.0 ± 7.7
3 h	7.9 ± 5.5	7.5 ± 3.9	6.6 ± 4.1
4 h	6.8 ± 5.1	5.3 ± 3.1	7.1 ± 6.9
6 h	5.3 ± 3.8	4.5 ± 3.2	5.5 ± 4.7
8 h	3.7 ± 3.2	3.7 ± 3.0	4.3 ± 4.2
12 h	2.2 ± 1.5	1.9 ± 2.0	3.2 ± 3.1

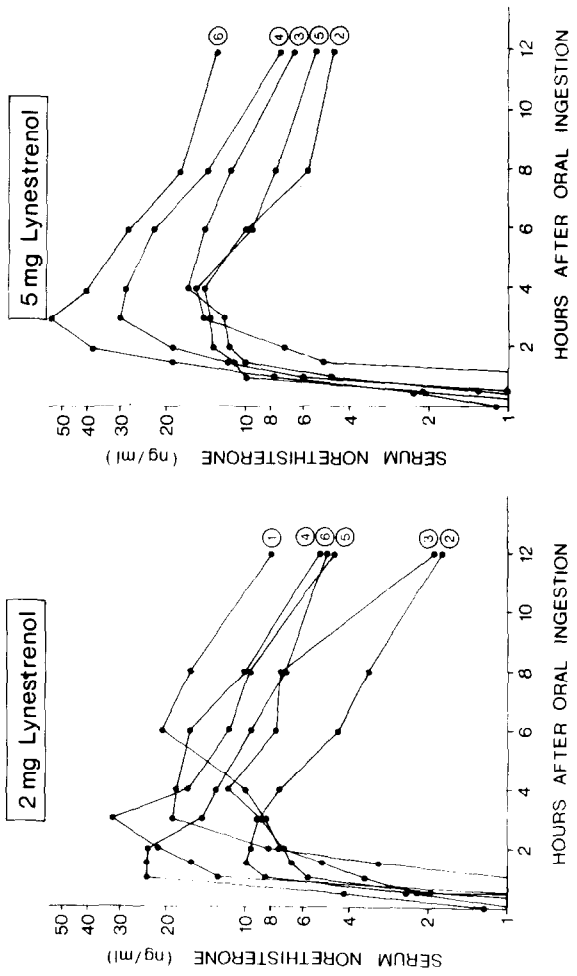

Fig.3. Serum NET concentrations in women following oral administration of 2 or 5 mg LYN.

The congruence of peak value, the AUC_{0-12}, and the half-life between both compounds indicates that 0.7 mg NET is bioequivalent to 1 mg LYN. These results correspond well to those of Odlind et al.(8).

Striking interindividual variations in the NET concentration curves after the ingestion of both NET and LYN had already been reported (8). Similar differences between individuals had also been observed when other hormonal formulations were investigated (9, 10). Back et al. (11) described, e.g., large variations in a concentration range of one or two orders when 50 µg ethinyl estradiol was orally administered in combination with 1 mg NET or with 0.5 mg Norgestrel. Twelve hours after ingestion, the serum level of NET ranged from 1.8 to 11 ng/ml, that of Norgestrel from 0.5 to 5.5 ng/ml, and that of ethinyl estradiol from 0.1 to 10 pg/ml. It is likely that these variations are due to individual differences in drug absorption, metabolism, distribution and elimination.

When the dose of LYN was raised to 2 or 5 mg, the individual peak values of serum NET generally increased, and tended to appear later, and the half-life was longer as compared to the administration of 1 mg LYN. This clearly indicates that the metabolic capacity of the liver is limited unless it is enhanced by means of enzyme induction during the continuous application of steroids.

A similar prolongation of the time interval between ingestion of NET or LYN, and between the maximal serum levels of NET was found by Nygren et al. (12) and Hümpel et al. (6). The latter author pointed out that the necessary conversion of LYN into NET could lead to competing side reactions which could result in the formation of polar metabolites with low elimination rates. As a consequence, the continuous application of these steroids may result in an accumulation of such polar metabolites.

When the serum level of NET subsequent to the ingestion of LYN was determined at different phases of the menstrual cycle, there appeared to be a trend towards a slowing down of the metabolic conversion and elimination. Even though the differences between means are statistically not significant, the cycle-dependent reduction in peak values and increase of half-life raise the intriguing question whether the reduced conversion of LYN is in some way related to the rising level of estradiol in midcycle, and of progesterone during the luteal phase. It is known that estrogens are capable of stimulating the synthesis of SHBG and thus elevate the serum level of this transport protein which could bind and protect the synthetic progestogen against enzymatic attack. This hypothesis is supported by the

observation that the continuous increase of NET peak values during the daily application of 1 mg NET and 50 μg Mestranol (10) is probably caused by the mestranol-stimulated increase in the production of SHBG. There are, however, no consistent changes of serum SHBG during the menstrual cycle (13). Therefore, estradiol and progesterone may interfere directly with the metabolization of LYN and NET by stimulating or inhibiting the action of various enzymes.

The contraceptive use of LYN, ethynodiol diacetate (1, 14, 15), mestranol (16), and other synthetic steroids which have to be convereted into a hormonally active metabolite can be problematic to a certain degree because the metabolic processes may be influenced by various factors. It may be assumed that pharmacokinetic parameters do not only affect the safety but also the severity of side effects of oral contraceptives. Moreover, the time concentration curves after ingestion of synthetic estrogens do not only seem to vary between individuals but also between different geographic locations (17, 18). The large interindividual variation in the serum level of synthetic steroids may also depend on environmental and constitutional factors (11). Both the liver and the intestines are involved in the processes of conversion, conjugation and elimination of steroids. Each of these metabolic steps can, e.g., be influenced by body weight, diet, diseases, stimulants, and drugs.

The dose-reduction in oral contraceptives which is currently en vogue brings about a certain risk of failure when the action of the steroidal components is interfered either by drugs or by disease processes affecting the rate of resorption. As a consequence, particularly women with inherently low serum levels of contraceptive steroids would thus be at an increased risk of a loss of contraceptive safety. In contrast, extremely high serum levels could as well be responsible for undesired side effects as it was the case in one of our volunteers. The results of the present study show clearly that the serum levels of NET after ingestion of NET or LYN can differ between individuals to such a large extent, that clinical consequences with respect to safety or side effects appear conceivable under certain circumstances.

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REFERENCES

1. Fotherby, K.: Metabolism of synthetic steroids by animals and man. Acta endocr. Suppl. 185: 119-142 (1974)

2. Rao, B.R., Wiest, W.G. and Allen, W.M.: Progesterone "receptor" in human endometrium. Endocrinology 95: 1275-1281 (1974)

3. Briggs, M.H.: Contraceptive steroid binding to the human uterine progesterone receptor. Curr. Med. Res. Opin. 3: 95-98 (1975)

4. Shapiro, S.S., Dyer, R.D. and Colás, A.E.: Synthetic progestins: in vitro potency on human endometrium and specific binding to cytosol receptor. Amer. J. Obstet. Gyn. 132: 549-554 (1978)

5. Kamyab, S., Fotherby, K. and Klopper, A.: Metabolism of (4-^{14}C)-lynestrenol in man. J. Endocr. 42: 337-343 (1968)

6. Hümpel, M., Wendt, H., Dogs, G., Weiß, C., Rietz, S. and Speck, U.: Intraindividual comparison of pharmacokinetic parameters of d-norgestrel, lynestrenol and cyproterone acetate in 6 women. Contraception 16: 199-215 (1977)

7. Mazaheri, A., Fotherby, K. and Chapman, J.R.: Metabolism of lynestrenol to norethisterone by liver homogenate. J. Endocr. 47: 251-252 (1970)

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

9. Weiner, E., Victor, A. and Johansson, E.D.B.: Plasma levels of d-norgestrel after oral administration. Contraception 14: 563-570 (1976)

10. Stanczyk, F.Z., Brenner, P.F., Mishell, D.R., Ortiz, A., Gentzschein, E.K.E. and Goebelsmann, U.: A radioimmunoassay for norethindrone (NET): measurement of serum NET concentrations following ingestion of NET-containing oral contraceptive steroids. Contraception 18: 615-633 (1978)

11. Back, D.J., Breckenridge, A.M., Crawford, F.E., MacIver, M., L'E. Orme, M. and Rowe, P.H.: Interindividual variation and drug interactions with hormonal steroid contraceptives. Drugs 21: 46-61 (1981)

12. Nygren, K.-G., Lindberg, P., Martinsson, K., Bosu, W.T. K. and Johansson, E.D.B.: Radioimmunoassay of norethindrone: Peripheral plasma levels after oral administration to humans and rhesus monkeys. Contraception 9: 265-278 (1974)

13. Anderson, D.C.: Sex-hormone-binding globulin. Clin. Endocr. 3: 69-96 (1974)

14. Walls, C., Vose, C.W., Horth, C.E. and Palmer, R.F.: Radioimmunoassay of plasma norethisterone after ethynodiol diacetate administration. J. Steroid Biochem. 8: 167-171 (1977)

15. Vose, C.W., Butler, J.K., Williams, B.M., Stafford, J.E. H., Shelton, J.R., Rose, D.A. and Palmer, R.F.: Bioavailability and pharmacokinetics of norethisterone in women after oral doses of ethynodiol diacetate. Contraception 19: 119-127 (1979)

16. Warren, R.J. and Fotherby, K.: Radioimmunoassay of ethynyloestradiol. J. Endocr. 63: 30p-31p (1974)

17. Goldzieher, J.W., Dozier, T.S. and de la Pena, A.: Plasma levels and pharmacokinetics of ethynyl estrogens in various populations. I. Ethynylestradiol. Contraception 21: 1-16 (1980)

18. Goldzieher, J.W., Dozier, T.S. and de la Pena, A.: Plasma levels and pharmacokinetics of ethynyl estrogens in various populations. II. Mestranol. Contraception 21: 17-27 (1980)