Upsala J Med Sci 89: 107–115, 1984

Enterohepatic Recirculation of Oestriol Studied in Cholecystectomized and Non-cholecystectomized Menopausal Women

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ABSTRACT

This study was done to evaluate the absorption of a single oral dose of 12 mg oestriol (Triovex®, Leo AB, Sweden) and to confirm the hypothesis that the enterohepatic recirculation can prolong the plasma oestriol elevation obtained.

Twelve menopausal women, six of whom had been cholecystectomized earlier, were given 12 mg oestriol orally. Fatty meals were given immediately after drug administration and then at four hourly intervals. Fat was given to induce the bileflow and provide oestriol to the intestine for deconjugation and enterohepatic recycling. One of the non-cholecystectomized women also remained fasting for 24 hours after oestriol administration.

Plasma concentrations of unconjugated oestriol were measured by a specific RIA-method.

In all women the plasma oestriol levels were considerably elevated for 24 hours. In the non-cholecystectomized women the plasma oestriol levels fluctuated in relation to meals whereas in the cholecystectomized women the fluctuations were not as pronounced, indicating that the release of biliary oestriol metabolites is the source of intestinal degradation and reabsorption to the systemic circulation. Fasting also gave increased and stable plasma oestriol levels.

After a high oral dosage of oestriol, the enterohepatic recycling renders oestriol an enhanced potency since the plasma oestriol elevation time is prolonged.

INTRODUCTION

A link between oestrogens and carcinomia of the endometrium has been claimed for many years (15,16). This has stimulated a reevaluation of oestriol in oestrogen therapy as oestriol is considered a short acting and therefore a weak oestrogen (8,14). In earlier studies (9,11) we have shown that the dosage and also the route of administration of oestriol are of importance. Enterohepatic recirculation is possibly also a matter of essential value in oestrogen metabolism, a result being that the plasma oestriol elevation is prolonged. The most important consequence of the enterohepatic recirculation is delayed final elimination of steroids from the body (3).

The nuclear binding time of oestriol is short and the nuclear receptor has to be occupied for a sustained period of time for full oestrogenic receptor stimulation (4,5). The current oral single dose regimen using low dosage of oestriol provides plasma oestriol elevation for less than six hours and probably does not induce full oestrogenic response.

The aim of this study was to prove the hypothesis that the dose and the enterohepatic recirculation of oestriol might be of importance for the plasma oestriol elevation time and hence the oestrogenic effect of oestriol.

Twelve mg oestriol was given orally to twelve menopausal women, six of whom had been cholecystectomized earlier. Fat-rich meals were given immediately after drug administration and then at four hourly intervals. Moreover, the oestriol elevation during a 24-hour fasting period was studied in one of the non-cholecystectomized women.

Plasma concentrations of unconjugated oestriol were measured at frequent intervals during 24 hours, using a RIA-method.

MATERIAL AND METHODS

Twelve healthy women who had been menopausal for at least two years volunteered for the study. Six of the volunteers had been cholecystectomized earlier. None of the twelve women had been on oestrogen therapy during the last two months. All the participants were of normal weight, height and had normal liver-function tests (S-ASAT, S-ALAT, Bilirubine, Alkaline phosphatase).

Twelve mg oestriol (Triovex®, Leo AB, Sweden) was given orally to all participants after a night-long fasting period and thereafter three meals containing a large amount of fat were given at four hourly intervals.

In addition, one of the non-cholecystoectomized women was given 12 mg oestriol orally (Triovex® Leo AB, Sweden) but remained fasting for another 24 hours.

Peripheral venous blood samples were collected at frequent intervals during 24 hours. One peripheral blood sample was drawn before and then every hour during 12 hours and finally at 24 hours after the oral administration of oestriol.

Plasma concentrations of unconjugated oestriol were measured by a specific radioimmunoassay (RIA) (8). Plasma concentration curves ($AUC_{0\text{-}12\ hours}$) ($AUC_{0\text{-}24\ hours}$) were calculated.

For statistical analysis, Wilcoxon's non-parametric test was used. The area under the observed plasma concentration time curve was calculated using the trapezoidal rule.

RESULTS

The mean values of plasma oestriol levels achieved after oral administration of 12 mg oestriol to six non-cholecystectomized and six cholecystectomized women, respectively, are presented in Fig 1.

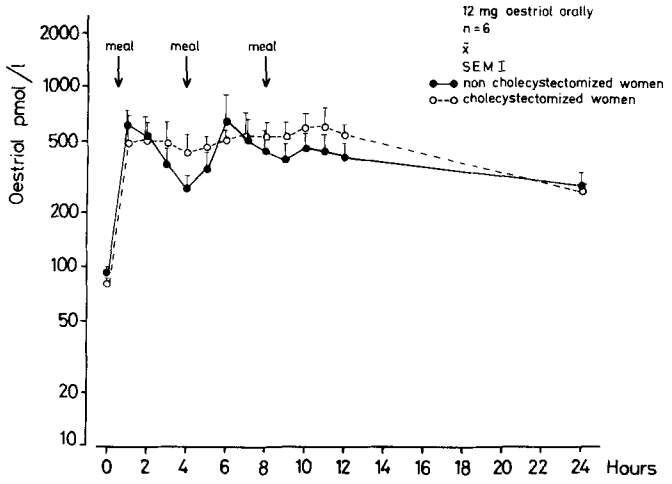

Fig 1 Plasma levels of oestriol after oral administration of 12 mg oestriol to six cholecystectomized women (broken line) and to six non-cholecystectomized women (unbroken line). Mean of the subjects and standard error of the mean is calculated.

Table 1 Comparison of AUC_{0-12h} and AUC_{0-24h} after oral administration of 12 mg oestriol.

Patient	AUC_{0-12h} not-op	op	AUC_{0-24h} not-op	op
1	11512.5	4377.5	17662.5	7767.5
2	4177.5	3085.5	9427.5	5455.5
3	3692.5	7702.5	6122.5	14062.5
4	3578.0	11622.5	7958.0	17532.5
5	6708.0	3750.5	11988.0	8550.5
6	2421.0	5032.5	4119.0	10942.5
Mean	5348.3	5928.5	9546.2	10718.5
	N.S.		N.S.	

A rapid and high increase in plasma oestriol levels is seen during the first hour in both groups. The increase in plasma oestriol then remains high during the first twelve hours and is still elevated 24 hours after drug administration.

In the non-cholecystectomized women there is an initial plasma oestriol elevation for about four hours and then a second and possibly a third elevation immediately after the meals.

In the cholecystectomized women the diurnal plasma oestriol elevation is comparative with that measured in the non-cholecystomized group, although the fluctuations related to meals are not as pronounced. When the area under the curve ($AUC_{0-12 \text{ hours}}$ $AUC_{0-24 \text{ hours}}$) was calculated (Table 1) the plasma oestriol elevation did not differ significantly between the two groups of women.

Figs. 2-3 illustrate the plasma oestriol levels in each subject in the non-cholecystectomized and the cholecystectomized groups. The subjects are referred to as numbers one to six in each group. From the figures it is evident that the inter-individual oestriol absorption as reflected by plasma oestriol elevation is considerable in both groups. The tendency in the plasma oestriol pattern, however, is different in the two groups of women.

In the non-cholecystectomized group (Fig. 2) most subjects had plasma oestriol fluctuations related to meals.

In the cholecystectomized group (Fig. 3) the inter-individual variations are more pronounced and the plasma oestriol fluctuations are not as related to meals.

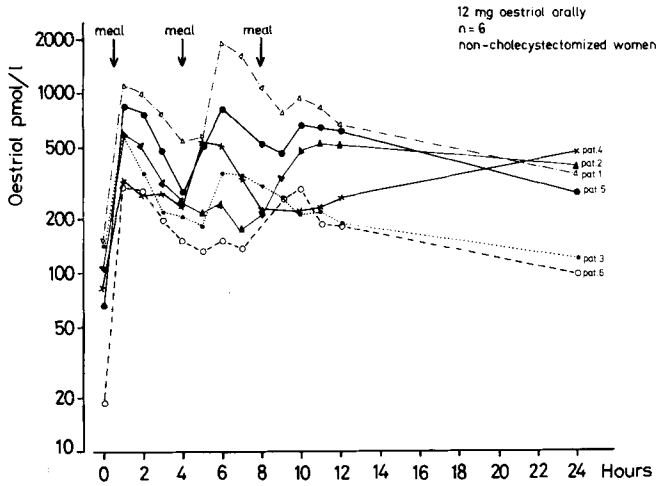

Fig. 2 Plasma levels of oestriol after oral administration of 12 mg oestriol to six non-cholecystectomized women.

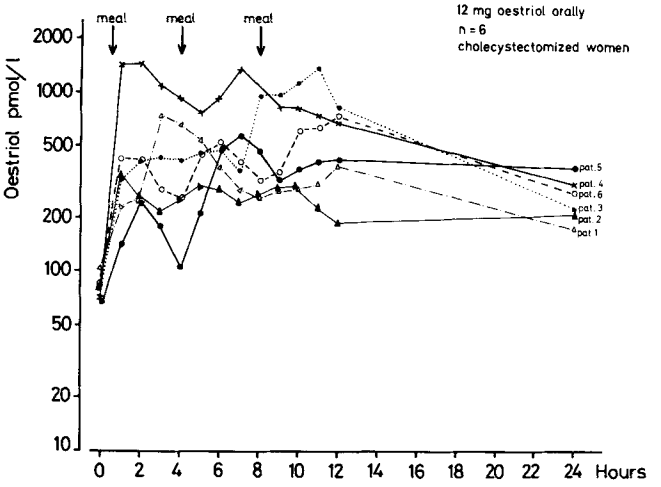

Fig. 3 Plasma levels of oestriol after oral administration of 12 mg oestriol to six cholecystectomized women.

Fig. 4 shows subject number five in the non-cholecystectomized group. The unbroken line illustrates plasma oestriol levels after administration of 12 mg oestriol orally followed by three fatty meals at four hourly intervals. An initial high plasma oestriol elevation is seen to last for about four hours followed by a second and third plasma oestriol elevation subsequent to the given meals.

The broken line illustrates the plasma oestriol levels after oral administration of oestriol with the subject fasting for 24 hours. During fasting there is an initial high plasma oestriol elevation, then the plasma oestriol level remains high and is higher at 24 hours when fasting than when eating.

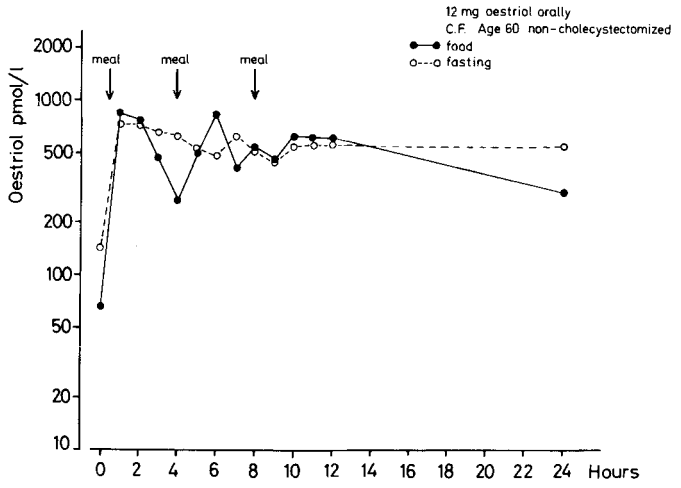

Fig. 4 Plasma levels of oestriol after oral administrtion of 12 mg oestriol to subject number five in the non-cholecystectomized group of women. The broken line indicates fasting while the unbroken line indicates the plasma oestriol level when meals are given.

DISCUSSION

Our data indicate that the amount of oestriol when given orally is of significant value as with a high dosage regimen the plasma oestriol levels are elevated for a prolonged period of time and do not reach pretreatment values within 24 hours (Figs. 1,2,3,4). This prolonged plasma oestriol elevation most possibly depends on enterohepatic recirculation of oestriol.

Our results revealing a prolonged plasma oestriol elevation after a single oral high dose of oestriol possibly is caused by the reappearance of oestriol in plasma from the enterohepatic circulation as the initial plasma oestriol elevation markedly declines in about four hours.

The plasma oestriol elevation pattern is fluctuating and the time interval between the peaks gives an indication of the enterohepatic recycling time. As illustrated in Fig. 4, the circulation time seems to be faster when eating than when fasting. This possibly depends on a more continuous bile release from the liver during fasting. The appearance of oestriol metabolites in the intestine is therefore also more continuous and may explain the prolonged and stable plasma oestriol elevation and a considerably high level still at 24 hours.

When eating (Fig. 4), the fluctuations in plasma oestriol are related to meals and most probably depend on the gallbladder's function as a reservoir with episodic release of biliary oestriol metabolites. These metabolites secreted to the intestine then probably are the source of degradation and reabsorption to the blood and are reflected as new plasma oestriol elevations. This theory is supported by the difference in plasma oestriol patterns found in our study between the non-cholecystectomized and cholecystectomized women. In the cholecystectomized women with a more continuous release of bile from the liver to the intestine the plasma oestriol elevation is more stable than in the non-cholecystectomized women with an episodic release of bile related to meals.

Our data agree with earlier reports that oestriol seems to be distributed between two pools when administered orally, the first being the systemic circulation and the second the enterohepatic circulation (13).

The oestrogens are metabolized mainly in the liver. All oestrogen metabolites are readily conjugated with glucuronic acid or sulfuric acid, and even double conjugates such as oestriol-3-sulfate-16α-glucuronide are formed. The latter are the most abundant conjugates in the bile (7,2). About a third to one-half of the circulating oestrogens are secreted in the bile and from this fraction 80 per cent is reabsorbed after hydrolysis in the intestinal tract (1).

In addition to the enterohepatic recirculation also the intestinal microflora as well as the mucosal cell metabolism must be considered as important for the metabolism and reabsorption of oestriol from the intestine.

Hill and co-workers (1971) have found that persons on a high-fat diet have a higher proportion of active anaerobic bacteria in the intestinal microflora and

secrete more biliary steroids than those on a low fat diet. The anaerobic bacteria are able to synthesize oestrogens such as oestradiol and oestrone from these steroids (6,12).

The association between diet and oestrogen metabolism is suggested also by other authors (10) who have found in vegetarian women a decreased ability of the intestinal microflora to deconjugate biliary oestrogen conjugates, which is a step necessary for their reabsorption. There is also a reduced β-glucuronidase activity in faeces of vegetarians, indicating a reduced enzymatic degradation of steroid metabolites.

Our knowledge of oestrogen metabolism has increased considerably during recent years but the results also have revealed a very complicated mechanism involving metabolism in the liver and in the intestine, the enterohepatic recirculation and the dependence of food habits. Further studies on the enterohepatic recirculation and on the intestinal metabolism have to be made to enhance our understanding of oestrogen metabolism

CONCLUSION

The amount of orally administered oestriol as well as food habits seem to be of importance for the plasma oestriol levels obtained in oestriol treatment.

In high dosage therapy, the enterohepatic recycling is of essential value by prolonging the plasma oestriol elevation time, which renders oestriol an enhanced potency as the receptor binding time also will be prolonged and full oestrogenic effect can be induced.

ACKNOWLEDGEMENT

This study was supported by the Swedish Medical Research Council (B 84 - 03 X - 03495 - 13 B). Statistical assistance was given by Leo AB, Sweden.

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