

Role of Diet on the Enterohepatic Recycling of Estrogen in Women Taking Contraceptive Pills

Pages with reference to book, From 213 To 215

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Abstract

The effect of diet on the enterohepatic recycling of estrogen after oral administration of 1 mg non-radioactive estriol (E3) was studied in six women using contraceptive pills. The women were followed for two consecutive menstrual cycles, firstly on a high fibre diet (HFD) and then changing on to low fibre diet (LFD) during the next cycle, hence each subject acted as its own control. The extent of enterohepatic recycling of estriol (E3) during the early follicular phase of menstrual cycle was assessed by monitoring during 48 hours, the urinary excretion of its two major metabolites, i.e., estriol-3-glucuronide (E3-3-G) and estriol 16 α -glucuronide (E3-16a-G). An increase in values of the variables including E3-3-G/E3-16a-G output ratio, E3-3-G output as % of total (E3-3-G+E3-16a-G) excretion and total E3(E3-3-G+E3-16a-G) output as % dose was noted as a result of change from high to low fibre diet. The urinary excretion of E3 in the form of its metabolites was also delayed as a result of dietary change. These findings reveal that extent of enterohepatic recycling of estrogen containing contraceptives could be higher in women on LFD as compared to those who are on HFD (JPMA 44:213, 1994).

Introduction

Natural and synthetic estrogen and progestin, produced endogenously or administered exogenously are excreted to a considerable extent in bile, enzymatically catalysed transformation in the gut and partly reabsorbed from the intestine. E3-16a-G and E3-3-G are regarded as the major metabolites of E3, of which E3-3-G has been suggested as being a purely gut metabolite whereas E3-16a-G as both gut as well as liver metabolite'. Any change in the level of E3-3-G with respect to E3-16a-G, might reflect the variation in the extent of gut hydrolysis and reconjugation of administered E3 and hence the extent of its enterohepatic recycling. The physiological role of this enterohepatic recycling of steroid hormones is largely unknown. One of the important consequences of the enterohepatic circulation is its delaying effect on the final elimination of steroids from the body. In addition, the product of intestinal metabolism may be biologically more active than those excreted via bile¹. Factors influencing the production, metabolism and extent of enterohepatic recycling of estrogen include body weight and its composition², nature of intestinal microflora³, antibiotics⁴ and other drugs^{5,6}, nature of diet⁷ and disease states⁸. Environmental and epidemiological aspects of estrogen dependent conditions strengthen the relationship between the diet and the estrogen status of women. Nutritional status and diet are known to influence the production and excretion of estrogenic hormones in women⁷. In this study the changes in the extent of enterohepatic recirculation of estrogen as a result of dietary change in women using contraceptive pills have been studied to see the role of dietary fibre on the enterohepatic recycling of estrogen and estrogen level in these women.

Subjects and Methods

Subjects

After informed consent 6 women taking contraceptive pills (“Nonnigest” which contains 0.5 mg norethindrone and 0.035 mg ethinyl estradiol) for the last 2-4 months were studied. Those with history of thrombophlebitis, thromboembolism, known or suspected estrogen dependent tumour (mammary or genital carcinoma), undiagnosed irregular vaginal bleeding, heart, kidney or liver disease or hypertension were excluded. All the subjects were followed for two consecutive menstrual cycles firstly while on high fibre diet (HFD) and then changing onto low fibre diet (LFD) during the next cycle. They took HFD (pre-weighed quantities) for three weeks back from the date of completion of urine collection in the first cycle and LFD (pre- weighed quantities) for three weeks back from the date of completion of urine collection in the second cycle. The mean± SEM values of age, body mass index and the dietary fibres and calories intake of the subjects were 24.30±0.50 years, 22.83±0.33 kg/M², 34.99±0.50g/day (HFD), 19.94±0.60g/day (LFD), 2199±30.95 K. cal/day (HFD) and 2200±66.57K. cal/day (LFD) respectively. High fibre diet provided about 35 grams of fibre and 2200 K. cal/day while low fibre diet provided about 20 grams of the fibre and 2200 k. cal/day. High fibre diet comprised mainly of chappatis (home made bread from dough on hot plate) and parathas (home made bread from dough on hot plate and fried in butter/vegetable fat) as the staples. Vegetables, meat and sometimes eggs were taken at the main meal. Milk in the form of tea and citrus fruits were also taken. Low fibre diet was almost isocaloric to high fibre diet and comprised mainly of sliced breads, buns and cakes (made from white flour). White rice was also included in the menu. Fruit intake was prohibited but cooked vegetables were allowed. There was sufficient intake of milk, meat and eggs. The mean fibre content and the calories for the diet were calculated from the values given in different tables^{9,10} on the basis of total dietary constituents taken over a week. All the participants within the same group took similar diet.

Collection of urine samples

First early morning urine samples were collected on fifth and the sixth day of menstrual cycle (day 1=1st day of bleeding). They were given 1 mg estriol (4x0.25 mg tablets ovestin) on the 6th day of the cycle and all urine samples voided over the next 48 hours (day 7, 8) were collected in separate plastic containers with the date and time of collection recorded. Urine samples were preserved with sodium azide (0.1g/100 ml urine) for storage and volume of each sample was noted before labelling its 10 ml aliquot. The aliquots were stored at -20°C until required for assay.

Urine analysis for estriol metabolites

Radioimmunoassay was used for the measurement of two metabolites of estriol, i.e., E3-3-G and E3-16a-G in urine using a modification of the method^{11,12}. 125[I] iodohistamides of E3-3-G and E3-16a-G (radioligands) were synthesized by the modification of the method of Maclean et al.¹³ using purified iodohistamine instead of iodination mixture containing iodohistamine. Assays were validated by using two groups of quality controls, one before and one after the assay. The intra-assay and inter-assay coefficient of variation were found to be less than 10% and 15% respectively.

Results

Urinary excretion of estriol metabolites E3-16a-G and E3-3-G is given in Table.

Table. Urinary excretion of estriol metabolites before and after estriol ingestion (1 mg) in women.

Variables	Values before estriol ingestion		Values after estriol ingestion	
	while on HFD	while on LFD	while on HFD	while on LFD
E ₃ -16a-G output (n.mole)	1.76 ±0.36 (6)	1.98 ±0.22 (5)	129.53 ±17.25 (5)	204.56* ±23.86 (6)
E ₃ -3-G output (n.mole)	0.11 ±0.03 (4)	0.53* ±0.11 (5)	7.52 ±1.92 (5)	17.56* ±3.29 (6)
E ₃ -3-G/E ₃ -16a-G Output ratio	0.06 ±0.03 (4)	0.30* ±0.09 (5)	0.05 ±0.01 (5)	0.08* ±0.01 (6)
E ₃ -3-G output As % of total (E ₃ -3-G=E ₃ -16a-G) excretion	9.76 ±4.21 (5)	18.90 ±5.11 (6)	5.21 ±0.63 (5)	7.66* ±0.76 (6)
Total E ₃ (E ₃ -16a-G+E ₃ -3-G) Output as % of dose	-	-	3.96 ±0.55 (5)	6.41* ±0.77 (6)

Values are given for two consecutive menstrual cycles, firstly while the subjects were on HFD and then changing on to LFD during the next cycle. Each value is the mean±SEM of number of observations shown in parentheses.

*P<0.05 as compared with HFD. HFD = High fibre diet, LFD = Low fibre diet.

The variables taken into consideration include E₃-16a-G output, E₃-3-G output, E₃-3-G/E₃-16a-G output ratio, E₃-3-G output as % of the total (E₃-3-G+E₃-16a-G) excretion and total E₃(E₃-16a-G+E₃-3-G) output as % of dose. The Values are given for two consecutive menstrual cycles, while the subjects were on high and low fibre diet. All variables showed a significant increase (P<0.05) with the change of diet from high to low fibre intake. Before E₃ ingestion a similar trend was observed for the excretion of E₃ metabolites (which were obviously of endogenous origin) but the increase was statistically significant only for E₃-3-G output and E₃-3-G/E₃-16a-G output ratio (P<0.05). The E₃-16a-G excretion was seen between 11-14 hours and second (35.99±8.76) between 20-23 hours at night.

A third (35.70 ± 5.91) appeared between 5-8 hours in the next morning. An elevation of urinary levels was observed between the 14-20 hours of the second thy after which there was a sudden fall in the urinary output of E3-16a-G.

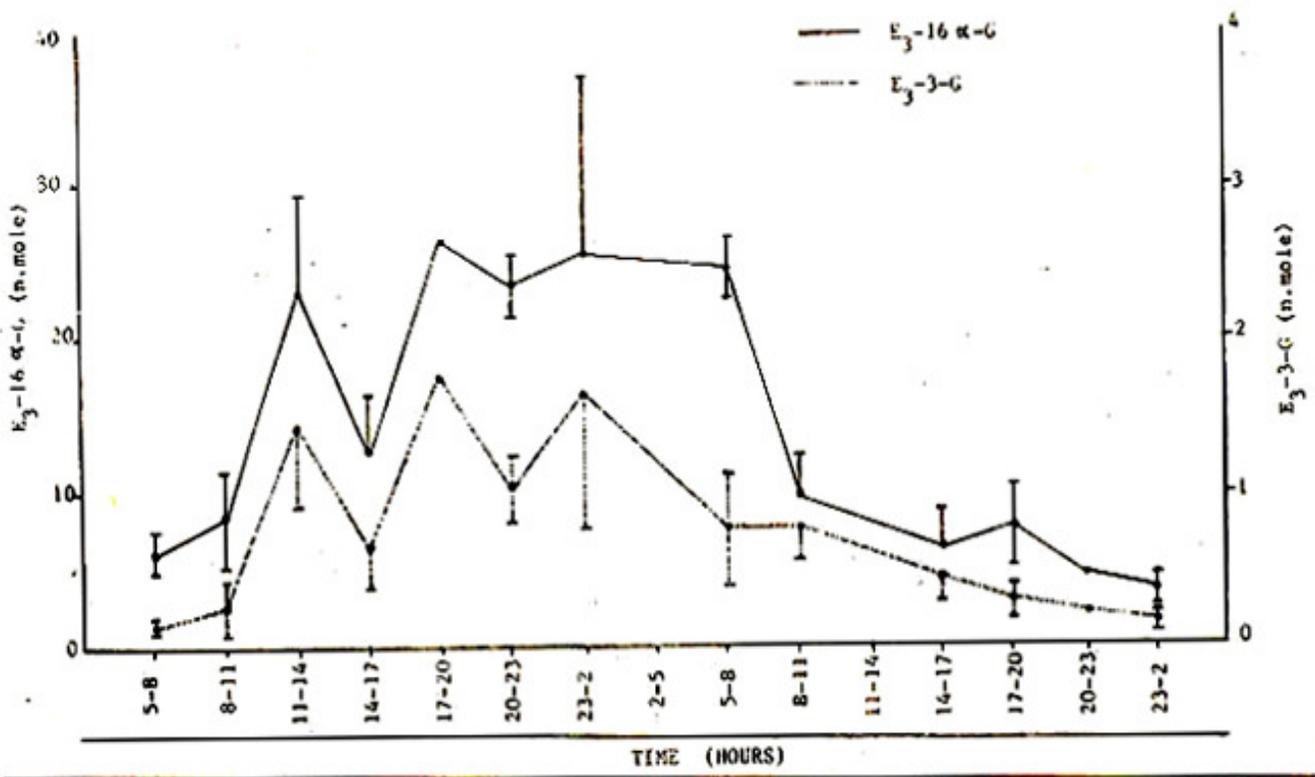


Figure 1. Diurnal variation in the urinary excretion of estriol metabolites in women on high fibre diet.

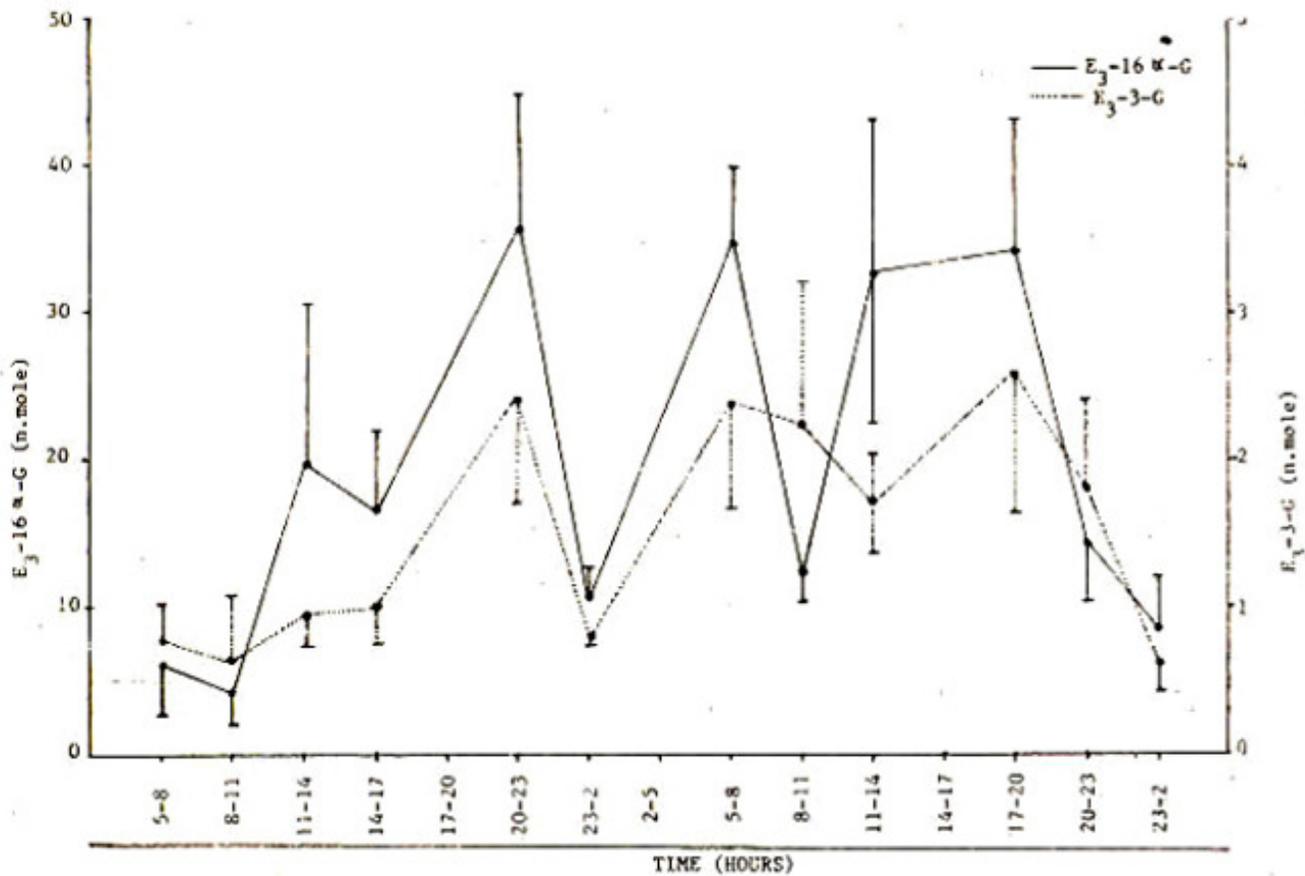


Figure 2. Diurnal variation in the urinary excretion of estriol metabolites in women on low fibre diet.

Figures 1 and 2 show a diurnal variation in the urinary excretion (n.mole) of estriol metabolites during 48 bouts after the ingestion of the drug. On HFD, E3-16a-G excretion showed two distinct peaks, first (23.18 ± 6.04) between 11-14 hours and a second (26.55) between 17-20 hours. The urinary levels remained elevated between 20-28 hours after which there was a sudden drop (between 8-11 hours) and no distinct peak was seen later. On LFD the first peak (19.60 ± 11.05) for E3-3-G excretion on HFD showed first peak (1.43 ± 0.5) between 11-14 hours followed by two peaks between 17-20 hours (1.76) and 23-2 hours (1.66 ± 0.89), it then showed a gradual fall. On LFD, the first peak (2.44 ± 0.74) of E3-3-G excretion was seen between 20-23 hours, a second on (2.38 ± 0.71) between 5-8 hours in the next morning and the final peak (2.58 ± 0.92) between 17-20 hours. Distribution of peaks for E3-16a-G and E3-3-G excretion in figures 1 and 2 shows that with the change from high to low fibre diet the final elimination of the estriol in the form of its metabolites was delayed.

Discussion

Our data indicates that changes in the fibre content of diet affect the estrogen metabolism, as the urinary excretion of orally administered estriol in the form of its metabolites (E3-16a-G and E3-3-G) was found to be higher when the subjects were on low fibre diet. There was difference between age and body mass index among the subjects. Their diet during the period of two menstrual cycles of study remained almost isocaloric but had different fibre content. Since E3-3-G has been suggested to be synthesized exclusively in the intestinal mucosal cells while E3-16a-G may also be synthesized in liver¹, the increase in variables such as E3-3-G output, E3-3-G/E3-16a-G output ratio and total E3

output as % dose reflect a higher extent of gut hydrolysis of biliary estrogen conjugates and reconjugation in the intestinal mucosal cells when the subject change onto low fibre diet. Before E3 ingestion a similar trend in the urinary excretion of E3 metabolites was noted with above dietary change. Urinary output of each E3 metabolite on either diet showed an appreciable difference before and after the use of tracer (1 mg non-radioactive estriol) which indicates that E3 remained as successful tracer in the present study. E3 has also been successfully tried as a tracer¹⁴ in cholecystectomized and non-cholecystectomized menopausal women to confirm hypothesis that the enterohepatic recirculation prolong the plasma estriol elevation obtained after oral dose. Low plasma estrogen levels have been reported in women consuming higher amount of dietary fibre and also in women taking oral antibiotics -both the conditions have been known to interrupt the enterohepatic recycling of estrogen probably by affecting the enzymes of intestinal mucosa and microflora^{7,15,16}. Previous studies^{1,7,17} on humans and animals have established that consumption of dietary fibre or administration of oral antibiotics can reduce foecal β -glucuronidase activity. β -glucuronidase activity has been reported to increase from the upper to the lower part of small intestine and is of both the bacterial and mammalian types. Diurnal variation in the urinary excretion of E3-3-G and E3-16a-G in our subjects indicated a more sustained estrogen level in the body when they were on LFD. Our findings reveal that high fibre content of the diet reduces the amount of estrogen in the enterohepatic circulation, whereas low fibre content increases it. This could be of importance in determination of efficacy of oral contraceptive preparations containing estrogens and structurally related substances.

Acknowledgement

Facilities extended by Dr. Talat Khan, Director, National Research Institute of Fertility Control, Karachi for carrying out radio immunoassays are gratefully acknowledged.

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