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Enterohepatic Recycling of Estrogen and its Relevance with Female Fertility

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Enterohepatic recycling of estrogen after oral administration of 1 mg non-radioactive estriol was studied in fourteen women selected as the control subjects and ten infertile women in whom the infertility was appearing to be of endocrine origin. The extent of enterohepatic recycling of estriol (E_3) during the early follicular phase of menstrual cycle was assessed by monitoring during 48 h the urinary excretion of its two major metabolites i.e; estriol 16 α-glucuronide (E_3-16 α-G) and estriol-3 glucuronide (E_3-3-G). The change in urinary level of E_3-3-G with respect to E_3-16 α-G was considered to reflect the extent of enterohepatic recycling of estriol. Lower values of urinary output of both metabolites in the infertile women as compared with the control subjects and the urinary excretion profile of both metabolites during 48 h after estriol ingestion reveal that the reduced extent of enterohepatic recycling could possibly be one of the factors which contribute towards the incidence of infertility in women.

Key words: Estrogen, Enterohepatic recycling, Infertility

INTRODUCTION

It has been reported that estrogen and progestin undergo enterohepatic recycling to an appreciable extent. Among the estrogen, E_3 exhibits the greatest enterohepatic recirculation. E_3-3-G and E_3-16 α-G are regarded as the major metabolites of E_3, of which the former has been suggested as being purely a gut metabolite whereas later as both liver as well as gut metabolite (Sandberg and Slaunwhite, 1965). Any change in the level of E_3-3-G with respect to E_3-16 α-G, might reflect the variation in the extent of gut hydrolysis and reconjugation of administered E_3 and hence the extent of its enterohepatic recycling.

One of the most important consequences of the enterohepatic recycling is the delaying effect it has on the final elimination of steroids from the body. In addition the product of intestinal metabolism may be biologically more active than those exceted via bile (Adlercreutz and Martin, 1980). Endocrine insufficiency is one of the major causes of infertility in women and the low levels of ovarian hormones may result from insufficient production or from the quicker elimination of ovarian hormones from the body (Ross *et al.*, 1981). In the present work the extent of enterohepatic recycling of estrogen has been studied in the infertile and in normal women with a view to investigate its relevance with female fertility.

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MATERIALS AND METHODS

Subjects

After informed consent and preliminary Medical examination 14 healthy women aged between 17-28 years (control subjects) and 10 infertile women aged between 25-35 years were studied. Those with history of thrombophlebitis, thromboembolism, known or suspected estrogen dependent tumor (mammary or genital carcinoma), undiagnosed irregular vaginal bleeding, heart, kidney or liver disease or hypertention were excluded. Infertile women had their plasma prolactin level within normal range, neither they had polycystic ovaries nor tubular defects, and their menstrual cycles were anovulatory as assessed by their L.H (Lutenizing hormone) and progesterone plasma peak levels. Mean ± SEM values of plasma peak level of prolactin, L.H. and progesterone of the subjects were 279.9 ± 23.59(mu/L), 8.69 ± 1.08(Iμ/L) and 5.99 ± 0.79(n.mole/L) respectively. Age, Body mass index, Dietary fiber intake and Calories intake of the infertile subjects had Mean ± SEM values as 28.8 ± 1.01(years), 22.59 ± 0.75(kg/M^2), 35.00 ± 0.30(g/day) and 2200 ± 18.66(K.cal/day) respectively. Those of the control subjects were found to be 21.7 ± 0.83(years), 21.80 ± 0.44(Kg/M^2), 35.15 ± 0.23(g/day) and 2200 ± 11.84

(K.cal/day) respectively. (Table I)

Both the groups of subjects were on usual Pakistani diet that comprised mainly of chappaties (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. Vegetable, meat and sometimes eggs were taken at the main meal. Milk in the form of tea and citrous fruits were also taken. The diet provided about 35 grams per day of the fiber and about 2200 K.cal/day. The mean fiber content and the calories for the diet were calculated from the values given in different tables (Chughtai and Khan, 1960; Khan and Eggum, 1978) on the basis of total dietary constituents taken over a week. All the participants with in the same group took similar diet.

Collection of urine samples

First early morning urine samples were collected by the subjects on the fifth and sixth day of menstrual cycle (day 1=1st day of bleeding. They were given 1 mg estriol (4 × 0.25 mg tablets, ovestin) on the sixth day of the cycle and all urine samples voided over the next 48 h (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

The urine analysis involved radioimmunoassay for the estimation of two metabolites of estriol i.e, E_3-3-G and E_3-16 α-G. using modification of the method of Baker et al, 1979.

^{125}[I] Iodohistamides of E_3-3-G and E_3-16 α-G (radioligands) were synthesized by modification of the method of Maclean *et al.*, 1981 using purified Iodohistamine instead of Iodina-

Table I. Age, body mass index, plasma peak level of Prolactin, L.H. and Progesterone and the dietary fibre and calories intake of the infertile women.

S.No.	AGE.	Body mass Index (Kg/M^2)	Prolactin (mu/L)	LH (lμ,/L)	Progesterone (n.mole/L)	Dietary fibre Intake (g/day)	Calories intake (K.Cal/day)
1.	30	24.8	275	7.8	4.6	33.90	2108
2.	27	24.0	220	9.0	5.3	36.35	2232
3.	25	25.3	234	10.6	9.7	33.55	2166
4.	25	25.1	340	11.0	2.8	34.50	2132
5.	35	21.3	410	5.3	2.4	35.50	2304
6.	28	22.5	210	6.8	6.6	35.20	2245
7.	28	22.6	190	4.6	7.8	34.68	2180
8.	28	22.7	235	16.4	9.0	34.60	2216
9.	29	19.0	365	8.7	4.5	35.78	2175
10.	30	18.6	320	6.7	7.2	35.64	2244
Mean ± S.e.m.	28.8 ± 1.01	22.59 ± 0.75	279.9 ± 23.59	8.69 ± 1.08	5.99 ± 0.79	35.00 ± 0.30	2200 ± 18.66

Table II. Urinary excretion of estriol metabolites (E_3-16α-G, and E_3-3-G) before and after estriol ingestion (1 mg) in the control subjects and the infertile women.

VariableS	Values before estriol Ingestion (mean of the values in EMU samples of 5th and 6th day of the cycle).		Values after estriol ingestion (sum of the values during 48 h after estriol ingestion)	
	Control Subjects.	Infertile Women.	Control Subjects.	Infertile Women.
E_3-16 α-G output (n.mole)	1.24 ± 0.14 (14)	1.30 ± 0.13 (10)	106.86 ± 7.79 (14)	74.41* ± 12.46(9)
E_3-3-G output (n.mole).	0.16 ± 0.02 (12)	0.14 ± 0.03 (9)	4.92 ± 0.64 (13)	2.66* ± 0.79 (8)
E_3-16 α-G /E_3-3-G output Ratio	9.19 ± 1.31 (12)	11.11 ± 2.78 (9)	22.35 ± 2.09 (13)	31.34 ± 6.10 (8)
E_3-3-G output as % of total (E_3-16 α-G+ E_3-3-G) excretioin	13.59 ± 2.17 (13)	10.53 ± 1.54 (9)	4.34 ± 0.29 (13)	3.52 ± 0.65 (9)
Total E_3 (E_3-16 α-G+E_3-3-G) output as % dose	-	-	3.11 ± 0.23 (13)	2.24 ± 0.38 (9)

Each value is the mean ± SEM of number of observation shown in parentheses.
*P<0.05 as compared with the control subjects.
EMU=First early morning urine.

tion mixture containing Iodohistamine. Assays were validated by running 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 E_3-16 α-G and E_3-3-G is given in the Table II. The variables taken into consideration include E_3-16 α-G output, E_3-3-G output, E_3-16 α-G/E_3-3-G output ratio, E_3-3-G output as % of total (E_3-16 α-G+E_3-3-G) excretion and total E_3(E_3-16 α-G+E_3-3-G) output as % dose.

The values of all the variables except E_3-16 α-G/E_3-3-G output ratio were found to be lower in the infertile women as compared with the control subjects but the difference was significant only for E_3-16 α-G output and E_3-3-G output ($P<0.05$).

E_3-16 α-G/E_3-3-G output ratio was apparently higher in the infertile women. Before estriol ingestion, the variables including E_3-3-G output and E_3-3-G output as % of total (E_3-16 α-G+E_3-3-G) excretion were apparently lower whereas E_3-16 α-G output and E_3-16 α-G/E_3-3-G output ratio appeared to be higher in the infertile women as compared with the control subjects ($P>0.05$).

Fig. 1 and 2 show a diurnal variation in the urinary excretion (n.moles) of estriol metabolites during 48 h after estriol ingestion in the control subjects and the infertile women respectively. In the control subjects first urinary excretion peak (23.27 ± 4.10) of E_3-16 α-G appeared between 20-23 h and the second one (15.46 ± 2.98)

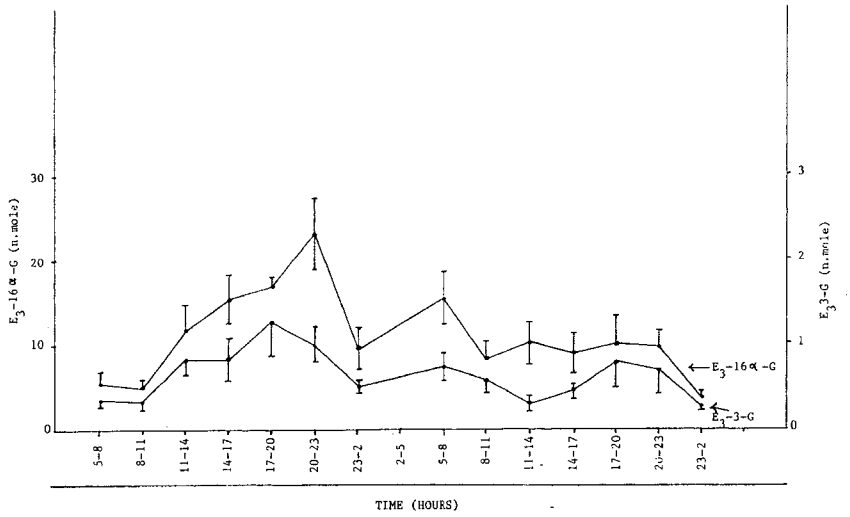

Fig. 1. Diurnal variation in the urinary excretion of estriol metabolites (E_3-3-G and E_3-16α-G) in the control subjects

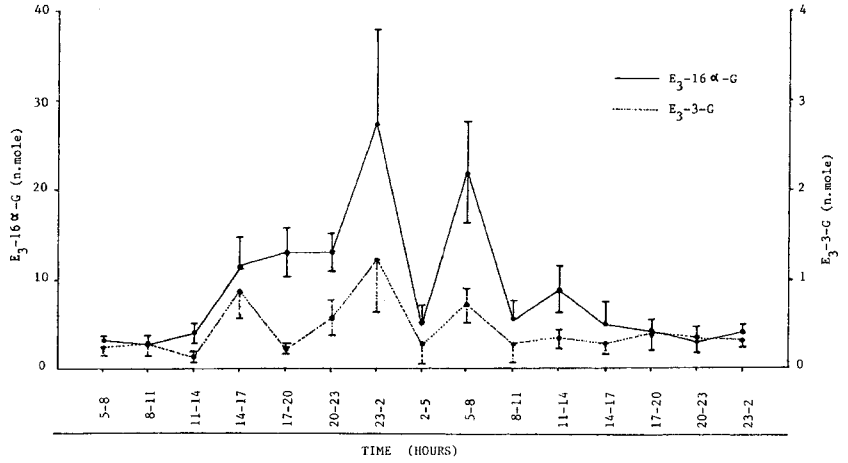

Fig. 2. Diurnal variation in the urinary excretion of estriol metabolites (E_3-3-G and E_3-16α-G) in infertile women

between 5-8 h, after which no distinct peak was seen. E_3-3-G excretion gave first peak (1.26 ± 0.37) between 17-20 h and second peak (0.73 ± 0.16) between 5-8 h. Its last peak (0.78 ± 0.28) appeared between 17-20 h of the next day. The time of the last (21.68 ± 5.56) peak of E_3-16 α-G urinary excretion in the infertile women and the control subjects was the same, but the last peak (0.71 ± 0.18) of E_3-3-G excretion in the infertile women appeared much earlier (between 5-8 h). The first (0.88 ± 0.29) and second (1.22 ± 0.57) peaks of E_3-3-G excretion in infertile women appeared between 14-17 h and 23-2 h respectively.

DISCUSSION

Results of the present study suggest a possible relevance of enterohepatic recycling of estrogen with female fertility, as the extent of urinary excretion of orally administered estriol in the form of its metabolites (E_3-16 α-G and E_3-3-G) was found to be low in selected infertile women. These women had neither polycystic ovaries nor tubular defects and their prolactin levels were within normal limits. In these women infertility was appearing to be of endocrine origin as the mechanical factors responsible for infertility were excluded. Endocrine insufficiency could either be due to under production of ovarian hormones or due to higher turnover rate or quicker elimination of ovarian products from the body (Ross *et al.*, 1981).

Since E_3-3-G has been suggested to be synthesized exclusively in the intestinal mucosal cells, while E_3-16α-G may also be synthesized in liver (Aldercreutz and Martin, 1980), the lower values of E_3-3-G output and E_3-16 α-G output in the infertile women as compared with the control subjects reflect a lesser extent of gut hydrolysis of biliary estrogen conjugates and then reconjugation in the intestinal mucosal cells. Apparently higher values of E_3-16 α-G/E_3-3-G output ratio and lower values of E_3-3-G output as % of total (E_3-16 α-G+ E_3-3-G) excretion, and total E_3(E_3-16 α-G+ E_3-3-G) output as % dose in the infertile women also support the above hypothesis. Before estriol ingestion the difference in the urinary excretion of estriol metabolites (during the early follicular phase of menstrual cycle) between the control subjects and the infertile women was sufficiently small and this can be expected so because of very low plasma and urinary levels of estrogen during this phase of the cycle.

Urinary output of each estriol metabolite in the control subjects and in the infertile women showed an appreciable difference before and after the use of tracer (1 mg nonradioactive estriol) which indicates that estriol remained as successful tracer in the present study. Estriol has also been successfully tried as a tracer (Heimer and Englund, 1984) in cholecystectomized and non cholecystectomized menopausal women to confirm hypothesis that the enterohepatic recirculation can prolong the plasma estriol elevation obtained after oral dose. Low plasma estrogen levels have been reported in women consuming higher amounts of dietary fiber (Goldin *et al.*, 1982) 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 (Goldin *et al.*, 1982; Gallagher *et al.*, 1980; Brewster *et al.*,1977).

Urinary excretion profile of estriol in the form of its metabolites in the control subjects and the infertile women during 48 h after estriol ingestion shows that last peak of E_3-3-G output appeared much earlier in the infertile women as compared with the control subjects (Fig. 1 and 2).

Since E_3-3-G level has been regarded as a good index of enterohepatic recycling of estrogen, it follows that there is a quicker elimination of estrogen from the body in the infertile women probably due to interrupted entrohepatic recirculation in these subjects. As the infertile women were on the diet almost identical in composition with that of the control group, the difference in the extent of enterohepatic recycling could possibly arise due to subnormal intestinal absorption of steroids which are available in lesser amount for enterohepatic recycling and excreted largely in feces. From the results of present study it can be speculated that in the infertile women the extent of enterohepatic recycling of endogenously produced active estrogen including more potent estradiol and estrone may also be low which would affect the estrogen status of the women and consequently may lead to the implications such as amenorrhea and infertility.

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