Urinary and Fecal Excretion and Incorporation of ^{14}C-Estradiol in Fat Depots of Obese and Lean Rats[1] (37560)

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Since estrogen is lipid soluble, claims have been made that body fat may remove circulating estrogen and prevent the rhythmic fluctuation of the hormone and thus interfere with menstrual and estrous cycles (1). Studies indicated that urinary excretion of a dose of radioactive estrogen was less in obese than in thin women suggesting that the body fat of the obese women provided a means of removing the exogenous estrogen. However, other reports indicated that retention of circulating estrogen by body fat is not sufficient to change the course of normal fluctuation and metabolism of the hormone. Twombly *et al.* (2) concluded by comparing blood plasma and fat depot estrogen that the latter site was not important in estrogen storage since plasma had a much higher concentration. Furthermore, according to Roncari (3) the release of estrogen from adipose tissues was very rapid and thus raised more doubts as to the importance of fat depots in the storage and indirectly on the metabolism of estrogen.

Our studies were therefore designed to determine the excretion in the feces and urine and the uptake by fat depots of radioactive estrogen. For this purpose, rats made obese by means of feeding a high fat diet were compared to normal weight rats produced by feeding a restricted amount of the high-fat diet or a grain diet.

Procedures. Twenty-one-day-old female Osborne-Mendel rats were fed a grain diet (4) or a high-fat diet *ad libitum*. The high fat diet contained 40% by weight Crisco instead of the 60% as previously decribed (5). The decrease in fat was made up by increasing sucrose. At 10 wk of age when the high-fat fed rats reached approximately 250 g body weight, part of the group of rats was then restricted to 8–9 g/rat/day of the diet so that body weight gain equaled that of the grain-fed rats. To assure adequate protein intake during restriction, the protein content of the diet was increased to 30% casein by decreasing the proportion of sucrose. The remaining high-fat fed rats continued on an *ad libitum* feeding regime. All rats had access to water at all times and were either housed singly in suspended wire cages or in metabolic cages for urinary and fecal collection. The rats were maintained at about 22° and in 12 hr each of light and darkness.

Six rats from each of the three dietary groups of animals at 29 wk of age were decapitated and parametric adipose tissues from each rat incubated with estradiol-4-^{14}C. At 41 wk of age, rats that had been fed the grain or the high-fat diet *ad libitum* were killed in a similar manner and parametric, perirenal and mesenteric adipose tissues were incubated with radioactive estradiol. In each case, duplicate or triplicate samples of 80–100 mg of tissues were incubated at 37° for 3 hr with 3 ml of Krebs-Ringer buffer, pH 7.4, containing 0.04 μCi of the estradiol, 6 mg glucose and 600 μg of porcine insulin.

Five rats from each of the three dietary groups were injected subcutaneously with the radioactive estradiol in corn oil according to body weight between the age of 30 and 40 wk. Five hours after injection, the rat was

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decapitated and perirenal, parametric, inguinal plus genital, mesenteric and perithyroid adipose tissues were removed, weighed and radioactivities determined.

In another experiment, five rats each from the three groups of animals between the ages of 36 and 38 wk were injected subcutaneously with 0.156 μCi/100 g body weight of radioactive estradiol dissolved in corn oil and urine and feces collected for 6 and 9 days, respectively. Urinary and fecal radioactivities were determined.

Estradiol-4-^{14}C (Amersham/Searle) was used in all experiments following purification by thin layer chromatography. The specific activity of the original compound was 51.4 mCi/m*M* according to supplier. Determination of radioactivities in samples (in duplicate or triplicate) as well as the purified estradiol used for incubation and injection was made with a Nuclear Chicago liquid scintillation-detection counter using ^{14}C as standards and channel ratio technique. All tissues were prepared for counting by dissolving in hyamine hydroxide except for fecal samples which were dried, ground and suspended in the scintillation fluid by means of Cab-O-Sil (Packard Instrument Company). All samples were counted to an accuracy of $\pm 2\%$ using the statistical method of Loevinger and Berman (4).

Only rats that had normal estrous cycles of 4–5 day duration as determined by vaginal smears were used in the study. All rats were in estrous phase at the time of decapitation for the *in vivo* studies and injection period for *in vivo* uptake and excretion studies.

Data were analyzed for statistical differences using analysis of variance.

Results and Discussion. Parametric adipose tissues from the rats fed the grain or high-fat diet to 29 wk of age incorporated estradiol to the same extent (29.7–32.4% of ^{14}C-estradiol added to medium/100 mg tissue). However, at 41 wk of age, significantly lesser proportion of the radioactivity was incorporated into parametric and mesenteric tissues of rats fed the high-fat than those fed the grain diet. Uptake of radioactivity by perirenal tissues was not influenced by diets (Table I).

When the radioactive estradiol was injected into the rat, the specific activity (dpm/100 mg tissue) was significantly higher ($p < 0.05$) in the mesenteric than the other adipose tissues whether the rat was fed grain or high-fat diet (Table II). Comparison of specific activities for a particular adipose tissue revealed that there was not much variation among the three dietary groups. The uniformity of specific activity for a given tissue might be species specific since according to Twombly *et al.* (2) obese patients had a lower adipose tissue specific activity than lean patients. Furthermore, since all rats were chosen for having normal estrous cycles, the uniformity in specific activities for a fat tissue could have been exaggerated.

Experimentation using obese and lean animals that have normal and abnormal estrous cycles will be useful in lending credence to the claim that obesity may decrease fertility by sequestering estrogen and in further support of the uniformity of specific activities. Since there are little differences in specific activity for a given tissue, animals having the greatest quantity of body fat had also the greatest retention of the injected radioactive estradiol (Table II). Thus, in general

TABLE I. *In Vitro* Incorporation of ^{14}C-Estradiol into Adipose Tissues of Rats Fed a Grain or High-Fat Diet *ad libitum*.

Diet	Adipose tissue		
	Parametric	Perirenal	Mesenteric
Grain, *ad libitum*	34.4 $\pm$ 2.7 (6)[a]	31.6 $\pm$ 2.9 (6)	36.6 $\pm$ 2.4 (4)
High-fat, *ad libitum*	28.4[b] $\pm$ 3.1 (6)	29.8 $\pm$ 2.6 (4)	31.5[c] $\pm$ 5.0 (7)

[a] The number of animals is indicated in parentheses. Values are averages $\pm$ standard errors expressed as % of ^{14}C-estradiol added in incubation medium per 100 mg tissues.

[b] Significantly different from grain-fed rats ($p < 0.01$).

[c] Significantly different from grain-fed rats ($p < 0.05$).

TABLE II. *In Vivo* Distribution of Radioactivity in Adipose Tissues of Rats.

	Grain, *ad libitum*			High-fat, *ad libitum*			High-fat, restricted		
Body wt (g)	280 ± 17			415 ± 38			294 ± 16		
^{14}C-estradiol injected (dpm/rat)	1,183,444			1,646,557			1,174,017		
No. of rats	5			5			5		
Tissues	Tissue wt (g)	Sp Ac (dpm/100 mg)	Injected dose (%)	Tissue wt (g)	Sp Ac (dpm/100 mg)	Injected dose (%)	Tissue wt (g)	Sp Ac (dpm) 100 mg)	Injected dose (%)
Perirenal	4.2[a] ± 0.9	145 ± 52	0.5 ± 0.2	26.7 ± 6.7	179 ± 35	3.0[b] ± 1.0	8.0 ± 1.9	154 ± 66	1.1[c] ± 0.6
Parametric	5.8 ± 1.1	209 ± 85	1.0 ± 0.4	21.9 ± 1.7	193 ± 31	2.7[b] ± 0.9	11.8 ± 2.4	216 ± 94	2.3 ± 1.4
Inguinal plus genital	5.3 ± 1.6	133 ± 38	0.6 ± 0.4	28.0 ± 4.1	158 ± 39	2.8[d] ± 0.9	9.8 ± 2.5	135 ± 48	1.2 ± 0.7
Mesenteric	4.6 ± 0.8	462 ± 198	1.8 ± 0.8	18.3 ± 3.7	266 ± 68	3.0[e] ± 1.0	5.2 ± 1.2	407 ± 281	1.8 ± 1.3
Perithyroid[f]	0.3 ± 0.1	147 ± 95	0.04 ± 0.04	2.3 ± 1.0	210 ± 73	0.3[d] ± 0.1	0.7 ± 0.3	152 ± 58	0.1[g] ± 0.04

[a] Mean ± standard errors.
[b] Significantly different from grain-fed rats ($p < 0.01$).
[c] Significantly different from high-fat ad libitum rats ($p < 0.01$).
[d] Significantly different from grain-fed rats ($p < 0.01$).
[e] Significantly different from grain-fed rats ($p < 0.05$).
[f] Adipose tissues surrounding thyroid.
[g] Significantly different from high-fat ad libitum rats ($p < 0.02$).

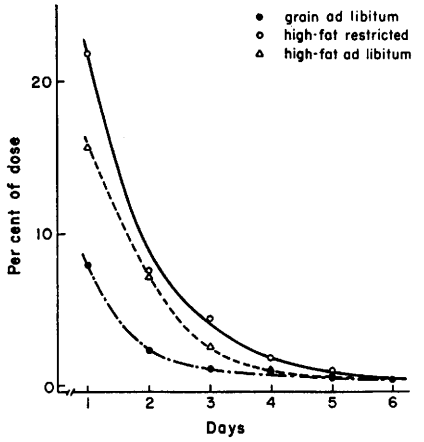

FIG. 1. Urinary radioactivities of rats injected with radioactive estradiol.

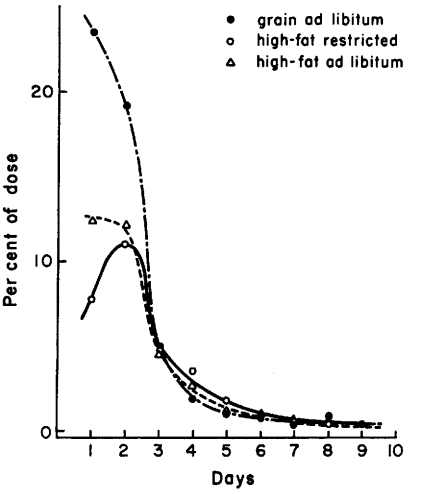

FIG. 2. Fecal radioactivities of rats injected with radioactive estradiol.

the percentage of the injected dose in each of the adipose tissues examined was greatest in high-fat fed rats followed in decreasing order by those fed high-fat restricted and the grain diet.

The rate of excretion of estradiol may also play a role in regulating estrous cycles. In the present experiment, the total radioactivity in feces plus urine was not significantly different among the three groups of rats but differed in the rate during the first few days after injection and in the route of excretion (Table III and Figs. 1 and 2). As a percentage of the injected dose the urinary excretion by the high-fat restricted rat was nearly three times that of the grain-fed rat, whereas, the high-fat *ad libitum* rat had an intermediate level (Table III). Fecal excretion expressed as a percentage of the dose was significantly greater for the grain-fed rats than for either the *ad libitum* or restricted high-fat rats. The difference in excretion whether in the urine or feces occurred mainly in differences of excretion rate in the first 2 or 3 days after administration of the radioactive estradiol (Figs. 1 and 2). After 2 or 3 days, differences in excretion disappeared. Thus, rats fed a high-fat diet had increased excretion of estradiol into the urine and decreased excretion into the feces for about 2–3 days after a dose of exogenous estradiol. The combined excretion, urinary plus fecal, was however, not affected by diets and therefore would not be expected to modified the body load of estradiol. However, since the route of excretion was altered by the diet the metabolism of estra-

TABLE III. Body Weight and Urinary and Fecal Radioactivities of Rats after Subcutaneous Injections of ^{14}C-Estradiol.

Diet	Body wt (g)	Dose/100 g body wt (dpm)	Urinary excretion[a] (% of dose)	Fecal excretion[b] (% of dose)	Total excretion (% of dose)
Grain, *ad libitum*	292[c] ± 27	343,075	12.6 ± 1.6	53.0 ± 6.0	65.6 ± 5.8
High-fat, *ad libitum*	464 ± 53	348,763	27.2[d] ± 1.8	34.7[e] ± 2.1	61.9 ± 1.8
High-fat, restricted	282 ± 13	348,409	36.7[d] ± 10.4	31.2[e] ± 8.1	67.9 ± 4.8

[a] Urine collection was 6 days.
[b] Fecal collection was 9 days.
[c] Mean ± standard errors of 5 rats.
[d] Significantly different from grain-fed rats ($p < 0.01$).
[e] Significantly different from grain-fed rats ($p < 0.01$).

diol was likely altered. Presumably the fecal excretion of estradiol was via the bile as conjugated estradiol whereas in the urine excretory products were metabolites of estradiol.

Summary. In vitro and *in vivo* uptake and fecal and urinary excretion of radioactive estradiol were determined in normally cycling rats fed a high-fat diet *ad libitum* or restricted or a grain diet *ad libitum* starting at 3 wk of age. At 29 wk of age, *in vitro* uptake of estradiol by parametric adipose tissue was not influenced by dietary treatment. However, at 41 wk of age, uptake per 100 mg of parametric or mesenteric adipose tissues was significantly less for high-fat fed rats than for grain-fed rats. *In vivo* uptake of injected estradiol-4-^{14}C was highest in the high-fat *ad libitum* rats followed by high-fat restricted and grain-fed rats. This differential uptake was due mainly to an increased adipose tissue size in the high-fat fed rats which were obese. Specific activities of tissues were not responsible for the different uptake. However, specific activities of tissue varied depending on the tissue examined. Thus, the mesenteric adipose tissue had the highest and the inguinal plus genital adipose tissue was the lowest. Total excretion of radioactivity was not different among the 3 dietary groups but the route as well as the proportion in the feces and urine varied significantly during the first 2–3 days after the injection of radioactive estradiol.

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