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EFFECTS OF ETHYNYL ESTRADIOL ON INCORPORATION OF [1-^{14}C] OLEATE INTO TRIGLYCERIDE AND KETONE BODIES BY THE LIVER

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Hypertriglyceridemia in women has been reported to result from prolonged administration of a combined estrogen-progestin oral contraceptive.[1,2] The increase in concentration of serum triglyceride was proportional to the estrogen content of the drug.[3] In recent years several investigators observed that administration of estrogen alone to humans,[4] rats,[5-7] and birds[8] produced a hypertriglyceridemia. Since the liver is the major organ contributing to serum triglyceride in the postabsorptive state[9] and since isolated perfused livers from female rats treated with ethynyl estradiol secreted more triglyceride than did livers from control animals,[10] it was essential to study further the mechanism(s) by which ethynyl estradiol acted. We therefore investigated the incorporation of[1-^{14}C]oleate (18:1) into triglyceride and ketone bodies by perfused livers isolated from normal fasted female rats and from rats treated with ethynyl estradiol.

Material and methods

Virgin female rats of the Sprague-Dawley strain weighing 195-240 g at the time of sacrifice were obtained from the Charles River Breeding Laboratories, Wilmington, Mass. The animals were maintained in separate metabolic cages and were given ethynyl estradiol, 15 μg/kg body weight, for a period of 14 days as described previously.[10] Livers were removed from the rats 14-16 hours after deprivation of food, were placed in an apparatus[11] and cyclically perfused. The perfusion medium consisted of washed bovine erythrocytes[12] suspended in Krebs-Henseleit bicarbonate buffer, pH 7.4 containing 3 g purified bovine serum albumin[13] and 100 mg glucose/100 ml buffer. The hematocrit was 30% at the start of the experiment. After a period of equilibration a complex of bovine serum albumin and fatty acid (10 μc[1-^{14}C] oleic acid;

150 mg oleic acid) was prepared as described previously[14] and infused into the perfusion medium under experimental conditions identical to those utilized by Soler-Argilaga et al.[15] The experiment was maintained for a period of 3 hours. Aliquots of perfusate were obtained after 1 and 3 hours of perfusion. Samples of liver were taken at the end of the experimental period. Triglycerides and fatty acids were extracted from the erythrocyte-free perfusate and from the liver and purified by thin layer chromatography on silicic acid.[16] Chemical analyses of fatty acid and radioactivity in triglyceride, fatty acid and ketone bodies were measured as described previously.[15] Calculation of specific activity of precursor free fatty acid and incorporation into triglyceride and ketone bodies was reported earlier.[17]

Results and discussion

The increased rate of secretion of triglyceride by liver resulting from treatment of the rat with ethynyl estradiol[10] is accompanied by increased incorporation of [1-^{14}C]oleate into hepatic and perfusate triglyceride (Table 1).

Table 1

Effects of ethynyl estradiol on incorporation of [1-^{14}C]-oleate into triglyceride and ketone bodies by the liver

Group	Triglyceride		Ketone Bodies
	Liver	Perfusate	Perfusate
Control (4)	2.92±0.22	2.71±0.39	4.96±0.26
Ethynyl Estradiol (5)	6.16±1.25*	6.73±0.63†	0.75±0.18‡

Number of experiments is shown in parentheses. The values given are means ± SE and are μmoles of oleic acid (18:1) taken up/g liver/hr and incorporated into triglyceride or ketone bodies. Details of calculations are presented in reference 17. Statistics indicate significance of differences from controls.

*P<0.05

†P<0.005

‡P<0.001

In contrast, incorporation of [1-^{14}C]oleate into ketone bodies was inhibited approximately 80%. These differences in metabolic disposition of oleate were not due to differences in uptake of oleate by the liver. Uptake of the fatty acid was 19.7±1.9 and 18.8±2.0 μmoles/g liver/hr by livers from control and estrogen treated rats respectively. The exogenous free fatty acid supplied to the livers from estrogen treated rats appears to be chanelled preferentially into triglyceride at the expense of oxidative pathways. The estrogens may inhibit hepatic pathways for oxidation of fatty acid, thus making more fatty acid available for synthesis of triglyceride; alternatively, estrogens may stimulate the rate of biosynthesis

of triglyceride directly. Both actions may possibly be involved. The increased rate of secretion of triglyceride by the liver produced by ethynyl estradiol, regardless of mechanisms, was probably the result of a net increase in the rate of biosynthesis of triglyceride.

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