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ABSTRACT

Female rats 4 weeks old were fed diets including beef tallow or safflower oil in combination with sucrose or rice starch. At 8 weeks of age, half the rats were orally administered 2 μg of estrogen (mestranol) in 5 μl of safflower oil and half were fed the vehicle only. After 10 to 14 days of estrogen treatment, rats were fasted and exsanguinated. Alterations were found in weight gain, liver weights, and levels of various lipids in plasma and liver. Most lipid levels were influenced by an interaction of mestranol with one of the dietary factors. No changes were observed in blood clotting activity as measured by prothrombin time and levels of plasma fibrinogen.

INTRODUCTION

Oral contraceptives (OCs) are known to elevate plasma lipids and to increase thromboembolism in women (Mann et al. 1975). It is possible that certain dietary histories predispose women to thrombosis. Estrogens from OCs may act synergistically with certain dietary patterns. Plasma and liver lipids and blood clotting activity in rats are known to be influenced by the interaction of OCs with dietary fat (Tabacchi and Kirksey 1973). Plasma and liver lipids are known to be influenced by type of carbohydrate fed with various fats (Carroll and Bright 1965). The purpose of this investigation was to study liver and plasma lipid levels and blood clotting activity of young female rats treated with estrogen and fed diets of varied fat-carbohydrate composition.

MATERIALS AND METHODS

The experiment was designed as a 2×2×2 factorial with 5 or 6 rats to each of 8 treatment combinations. Experimental variables were the type of dietary fat, beef tallow (BT) or safflower oil (SO); the type of dietary carbohydrate, sucrose (S) or rice starch (RS); and estrogen treatment, with mestranol[1] (+M) or without mestranol (-M).

At 4 weeks of age, female rats of the Sprague-Dawley[2] strain were assigned at random to treatment groups. In a temperature controlled room, rats were housed individually in wire-bottomed cages where they had free access to water and ration (Table I). At 8 weeks of age, half the rats fed each of the 4 diets were orally administered 2 μg of mestranol, a common estrogen in OCs, in 5 μl of safflower oil and half were fed the vehicle only. This quantity of mestranol is approximately the minimum dosage of estrogen used in combination with a progestin to prevent conception in rats (Aftergood and Alfin-Slater 1971). In proportion to body weight, this dosage in rats is approximately 10 times the quantity of estrogen taken by women who use OCs.

After 10 to 14 days of estrogen treatment, rats were fasted for 4 hours, anesthetized with sodium pentobarbital, and exsanguinated from the abdominal aorta. Liver and plasma were assayed for lipid content. Triglyceride was assayed by the method of Mendez et al. (1975). The method of Zlatkis et al. (1953) was used to determine cholesterol. Phospholipid was hydrolyzed by the method of Youngberg and Youngberg (1930), and the inorganic phosphorus thus released was determined by the method of Fiske and Subbarow (1925). Plasma coagulation properties assayed were prothrombin time (Faulkner and King 1970) and level of plasma fibrinogen (Ratnoff and Menzie 1964). Data were examined by analysis of variance in a completely random design. Significance was determined from *F*-values.

[1]Amersham Searle Chemicals, Inc., Chicago, Illinois.
[2]Hormone Assay Laboratories, Chicago, Illinois.

RESULTS AND DISCUSSION

Mestranol depressed weight gain by 46% (Table II). Rats treated with mestranol not only ate less ration than did their counterparts, but also used food consumed less efficiently for weight gain. Rats fed SO had heavier livers in relation to body weight than did those fed BT. Relative liver weights of rats treated with mestranol were greater because of lower body weights. Values for liver lipids were stated per 100 mg of liver protein in order to make comparisons unaffected by liver or body weights.

Total liver lipids and liver triglycerides were altered by an interaction of the carbohydrate and estrogen factors (Fig. 1a, b). Without mestranol, liver lipid was greater when the dietary carbohydrate was RS rather than S. However, when the treatment included mestranol, RS was unable to elevate total liver lipids and triglycerides.

It is not clear why, in the absence of mestranol, RS but not S elevated liver lipids. Carroll and Bright (1965), who fed low carbohydrate-high fat diets, found that accumulation of liver lipids in male rats was dependent on both the type of carbohydrate and the type of fat fed. In that study, liver lipid was decreased when rats were fed fructose with corn oil rather than glucose with corn oil, or either carbohydrate with hydrogenated coconut oil.

The fate of RS in the presence of mestranol is unknown. Cortisol, which increases gluconeogenesis and inhibits fatty acid synthesis in the liver, is known to be elevated in plasma of rats treated with mestranol (Renaud 1970). Perhaps glucose from RS undergoes lipogenesis in the absence of mestranol but is utilized by cells or converted to glycogen in the presence of mestranol.

Liver cholesterol was elevated by SO and by the interaction of SO with RS, regardless of whether or not mestranol was administered (Fig. 1c). Plasma cholesterol was depressed by mestranol, an action independent of the type of fat or carbohydrate fed (Table III). Thus, the elevation of liver cholesterol and the depression of plasma cholesterol were unrelated and not the result of transfer from plasma to liver.

Plasma phospholipids were elevated by BT over SO, and depressed by the interaction of SO with mestranol. Although mestranol had no influence on plasma phospholipids when the dietary fat was BT, phospholipids were greatly decreased by mestranol when SO was fed (Fig. 1d). Aftergood and Alfin-Slater (1971) reported that plasma phospholipids were decreased in female rats fed a stock diet and treated with an OC.

Plasma cholesterol and plasma phospholipids of rats were depressed in this experiment by mestranol or by an interaction that included mestranol. Yet these lipids commonly are elevated in the plasma of women who take OCs. In contrast, plasma triglycerides seem to be elevated in both women and rats by most OCs and by estrogen. Although plasma triglycerides were not measured in this study, it is suspected that this lipid was elevated by mestranol (Kekki and Nikkila 1971, Tabacchi and Kirksey 1973, Kudzma et al. 1975). The liver of an estrogen-fed animal is likely to be overburdened with fatty acids because estrogen elevates plasma cortisol levels and

cortisol mobilizes fatty acids from adipose tissue. The liver, which cannot use fatty acids for gluconeogenesis, may incorporate them into triglycerides and transfer them to the blood as lipoproteins.

No difference in blood clotting activity among treatments was found as measured by prothrombin time or level of plasma fibrinogen. Tabacchi and Kirksey (1973) reported that OCs containing mestranol elevated levels of plasma fibrinogen in 7-month-old female rats when the diet included coconut oil or cholesterol. Negative results of the present experiment may have been due to the youth of the rats tested.

The results of this study indicate that young female rats undergo changes in liver and plasma lipids in response to the type of dietary fat or carbohydrate, and that these changes can be modified by administration of mestranol. Yet certain blood clotting factors do not appear to be altered either by mestranol or by dietary fat or carbohydrate.

Table I. Composition of Diets

	g/100 g diet
Vitamin-free casein[1]	19.6
DL-Methionine[1]	0.4
Salts, R. H.[2]	5.0
Vitamin Mixture[3]	0.3
Choline Chloride	0.2
Fat[4]	20.0
Carbohydrate[5]	30.0
Alphacel[1]	24.5

[1] ICN Pharmaceuticals, Inc., Cleveland, OH.

[2] Rogers, Q. P. and Harper, A. E. 1965. J. Nutr. 87: 267-273.

[3] mg/100 g of ration: thiamine HCl, 0.8; pyridoxine, 0.4; Ca pantothenate, 4.0; niacin, 5.0; inositol, 20.0; folic acid, 0.4; vitamin B_{12} (triturated 3000 μg per g), 1.33; biotin, 0.02; vitamin A powder (20,000 IU per g), 10.0; calciferol (850,000 IU per g), 0.18; DL-α-tocopherol powder (250 IU per g), 30.0; menadione, 0.38; riboflavin, 0.6; and sucrose, 176.89.

[4] Beef tallow or safflower oil.

[5] Sucrose or rice starch.

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Table II. Weight Gain, Food Intake, and Food Efficiency Ratio During Period of Mestranol Treatment

	Weight gain (g)	Food intake (g)	FER[2]
Fat			
BT[1]	10	90	11
SO	10	84	12
Carbohydrate			
S	9	86	10
RS	11	87	13
Estrogen			
-M	13	94	14
+M	7	80	10
Significant F values			
($P < 0.005$)	Est	Est	
($P < 0.05$)			Est

[1] BT = beef tallow, SO = safflower oil, S = sucrose, RS = rice starch, -M = without mestranol, +M = with mestranol.

[2] Food efficiency ratio = g weight gain/100 g food intake.

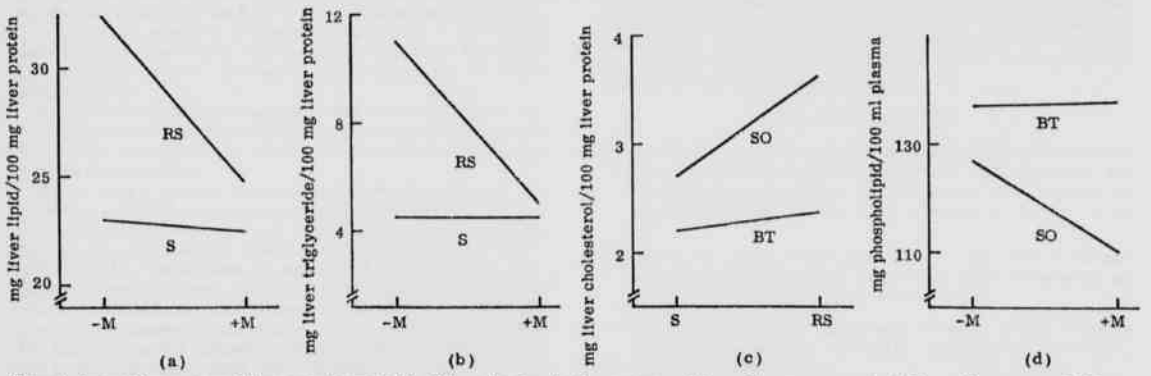

Figure 1. Interaction means of liver and plasma lipids. RS = rice starch, S = sucrose, -M = without mestranol, +M = with mestranol, SO = safflower oil, BT = beef tallow.

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Table III. Composition of Liver and Plasma Lipids

	Liver lipids			Plasma lipids	
	Total Lipid	Triglyc-eride	Choles-terol	Choles-terol	Phospho-lipid
	mg/100 mg liver protein			mg/100 ml plasma	
Fat					
BT[1]	24.67	6.80	2.29	95.1	137
SO	26.41	5.29	3.13	89.9	118
Carbohydrate					
S	22.78	4.55	2.45	94.0	128
RS	28.45	7.70	2.95	91.0	127
Estrogen					
-M	27.47	7.43	2.72	102.4	132
+M	23.68	4.75	2.67	83.1	124
Significant F values					
($P < 0.005$)	CHO, Est, CHO X Est	CHO, Est, CHO X Est	Fat, CHO,	Est	Fat
($P < 0.05$)			Fat X CHO		Fat X Est

[1] BT = beef tallow, SO = safflower oil, S = sucrose, RS = rice starch, -M = without mestranol, +M = with mestranol.