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Oral Contraceptives: Effects on Plasma Insulin Response to Glucose and on the Response to Insulin and 2-Deoxyglucose Uptake by Peripheral Tissue[1] (38819)

K. Y. LEI, M. G. YANG[2], D. OBERLEAS, AND A. S. PRASAD

Veterans Administration Hospital, Allen Park, Michigan 48101 and Department of Medicine, Wayne State University School of Medicine, Detroit, Michigan 48201

Impaired glucose tolerance (1), elevated fasting blood insulin (2), and increased insulin response to glucose (2) and arginine (3), have been reported in some women receiving oral contraceptives. Women with a family history of diabetes, a history of having delivered large babies, of gestational diabetes, glucosuria, or polyhydramios are said to be especially susceptible to the effect of oral contraceptives on carbohydrate metabolism.

When oral contraceptives were administered subcutaneously (4) or orally (5) to rats, impaired glucose tolerance was observed after 2 and 4 wk of treatment, respectively. Recently, Goldzieher (1) indicated that either the estrogen or the progestin component of the combination-type oral contraceptives can affect glucose metabolism in women, but the effect of the estrogen seems to be minor. In rats, norethynodrel, a 19-nor-progestin, was found most effective on glucose metabolism (6).

Steroid treatment decreases the effect of insulin on *in vitro* glycogenesis in diaphragm tissue and lipogenesis in adipose tissue of rats (6). It appears that the altered glucose metabolism may be the result of a peripheral resistance to insulin.

The studies described in this paper were conducted to establish whether peripheral tissue resistance to exogenous insulin exists *in vivo* and whether an enhancement of plasma insulin response to oral glucose occurs in contraceptive-treated rats. Furthermore, the experiments were designed to determine whether insulin resistance was due to a slower rate of glucose transport into tissues.

Materials and Methods. Eleven-week-old female Sprague–Dawley rats weighing between 250 and 270 g were used in three experiments. They were housed individually in suspended wire cages in a temperature- and light-regulated room. They were fed a nutritionally adequate diet containing 0.027 mg mestranol (an estrogen) and 1.83 mg norethynodrel (a progestin) per kilogram (7). Control rats were pair-fed the same diet without steroids. Since the diet intake was equalized, the two treatment groups showed comparable weight gains. The daily intake of steroids was approximately 0.1 mg of norethynodrel and 1.5 μg of mestranol/kg of body weight. These levels were comparable to those used by women for contraceptive purposes. Water was given ad lib. Other details are described in the captions of figures and tables.

In the first experiment, oral glucose tolerance was performed by force feeding glucose (300 mg/100 g body weight, in a 50% solution; w/v) to rats that had been fasted for 18 hr and had been anesthetized with sodium pentobarbital (5 mg/100 g body weight; subcutaneously). Blood samples were obtained by heart puncture for determinations of serum glucose, by a glucose oxidase method (8) and the serum insulin, by a modification of the method of Hales and Randle (9) (Insulin Immunoassay Kit, Amersham/Searle Corporation).

In the second experiment, the effect of the contraceptives on the insulin sensitivity of adipose tissue and diaphragm was studied.

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[2] Present address: Norwich Pharmacal Company, Norwich, New York 13815.

For these purposes, eight pairs of control and steroid-treated rats were injected intravenously with a tracer dose of glucose-U-^{14}C and 0.2 U insulin/kg body weight. Another eight pairs, injected with glucose-U-^{14}C but not insulin, served as controls. Fifteen minutes after the injection, the animals were decapitated. The diaphragm and parametrial adipose tissues were rapidly excised in order to measure the incorporation of ^{14}C into adipose tissue fatty acids, glyceride-glycerol and glycogen, and into diaphragm glycogen. The tissues were analyzed as described previously (6). Blood was collected for serum glucose, free fatty acids (10), and radioactive materials determinations.

The effect of contraceptive steroids on the *in vitro* uptake of 2-deoxyglucose-1-^{14}C (DOG) by peripheral tissues was investigated in the third experiment. Rats were decapitated and their hemidiaphragms obtained by dissecting through the xyphoid, central tendon, and vertebra, leaving intact the attachment of each hemidiaphragm to its half of the rib cage. Pieces of parametrial adipose tissue weighing about 100 mg were also excised. Tissue samples were placed in 25-ml Erlenmeyer flasks, precoated with silicone to prevent the loss of insulin by adsorption to the vessel wall. The concentrations of substrate and of insulin in the Krebs–Ringer bicarbonate buffer (pH 7.4) are indicated in Table II. The incubation was performed in a metabolic incubator at 37°C for 45 min. After incubation, the hemidiaphragms were washed three times in saline, the muscle tissue was dissected free, rinsed in saline three times, blotted after each rinse, and digested with Unisol (Isolab, Ohio) in a scintillation vial. "Aquasol" (New England Nuclear) was used as scintillant and the radioactivity was determined in a Tri-Carb Liquid Scintillation Spectrometer (Packard). All data were analyzed by analysis of variance.

Results. First experiment. Ten weeks of treatment, did not modify significantly the insulinogenic response to oral glucose (Fig. 1). However, the steroid treatment significantly decreased glucose tolerance (Fig. 1).

Second experiment. It was found that insulin stimulated the conversion of glucose into adipose tissue fatty acids, and glyceride–glycerol and into diaphragm glycogen, but not into adipose tissue glycogen (Table I). The levels of serum free fatty acids, glucose, and radioactive materials were significantly lower in the insulin-treated than the control rats (Table I).

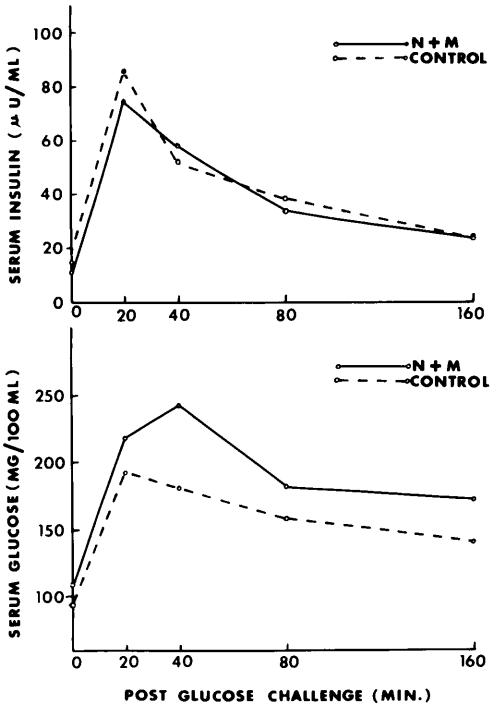

FIG. 1. Serum insulin and glucose levels during oral glucose tolerance test after 10 wk of norethynodrel plus mestranol (N + M) treatment. Broken line represents control group. Ten rats/treatment.

The effect of exogenous insulin on the conversion of blood glucose into fatty acids by the adipose tissue was significantly lower in the steroid-treated than in the control rats (Table I). There were no significant differences in serum glucose, free fatty acids, and radioactive materials, and in the conversion of blood glucose into adipose tissue glyceride–glycerol and into adipose tissue and diaphragm glycogen between steroid-treated and control rats whether injected with insulin or saline.

Third experiment. The results indicate that the uptake of DOG by the hemidiaphragm and adipose tissue was increased significantly as its concentration in the medium was increased (Table II). No significant differences were observed in DOG

TABLE I. EFFECT OF INSULIN ON SERUM GLUCOSE AND *IN VIVO* CONVERSION OF GLUCOSE TO VARIOUS PRODUCTS IN TISSUES OF ORAL CONTRACEPTIVE-FED AND CONTROL RATS[a]

Tissue	Product	Saline injected		Insulin injected		Analysis of variance (treatment comparisons)		
		Control	Contraceptive	Control	Contraceptive	Control vs contraceptive	Saline injected vs insulin injected	Interaction
Adipose	Fatty acids	8.2 ± 1.0[b]	7.7 ± 0.9	186.4 ± 73.9	35.4 ± 12.4	0.05	0.01	0.1
	Glyceride–glycerol (dpm/100 mg tissue)	166 ± 18	178 ± 25	1159 ± 232	837 ± 72	—	0.005	—
	Glycogen	22.9 ± 2.6	25.6 ± 3.7	24.6 ± 4.0	24.2 ± 2.2	—	—	—
Diaphragm muscle	Glycogen	346 ± 81	226 ± 44	1146 ± 121	1044 ± 169	—	0.005	—
Serum	Glucose (mg/100 ml)	79.3 ± 4.9	74.6 ± 2.3	48.3 ± 5.1	47.1 ± 2.2	—	0.005	—
	Radioactive materials (dpm/μmole glucose)	14144,± 837	14864 ± 1561	10514 ± 1241	13333 ± 1060	—	0.05	—
	Free fatty acids (μeq/liter)	598 ± 48	600 ± 61	266 ± 24	337 ± 28	—	0.005	—

[a] Contraceptive-fed and control rats were fasted for 12 hr and then injected intravenously 15 min prior to sacrifice with 24 μCi glucose-U-^{14}C/kg body weight and, where indicated, 0.2 U insulin/kg body weight.

[b] Mean for eight observations ± standard error of mean.

TABLE II. *In Vitro* Uptake of 2-Deoxyglucose-1-^{14}C by Hemidiaphragm and Parametrial Adipose Tissues in Oral Contraceptive-Fed Rats[a]

Tissue	Treatment	2-Deoxyglucose μmole/ml buffer[b] (nmoles/100 mg tissue/45 min)			
		0.5	1	2	4
Hemidiaphragm[c]	Control[d]	34.6 ± 2.0[e]	65.9 ± 3.5	121.1 ± 6.5	252.8 ± 25.6
	Steroid treated†	28.3 ± 1.8	57.6 ± 1.7	116.7 ± 6.5	206.2 ± 18.5
Adipose[c]	Control	11.8 ± 1.5	17.8 ± 2.2	35.8 ± 5.8	54.8 ± 9.9
	Steroid treated	8.2 ± 0.6	15.5 ± 0.9	25.7 ± 1.6	50.7 ± 3.2

[a] Six weeks of steroid treatment.

[b] Buffers for hemidiaphragms contained (per ml): insulin 300 μU, 2-deoxyglucose-1-^{14}C 0.1 μCi, and various levels of 2-deoxyglucose as indicated; buffers for adipose contained (per ml): insulin 10 μU, 2-deoxyglucose-1-^{14}C 0.1 μCi, and various levels of 2-deoxyglucose as indicated. Each incubation flask contained 4 ml of buffer for the hemidiaphragm and 3 ml of buffer for the adipose tissue.

[c] 2-Deoxyglucose concentration effect, linear response (degree of freedom = 1) $P < 0.005$.

[d] Main effect of control vs steroid treated (degree of freedom = 1) $P < 0.10$.

[e] Mean for nine rats ± SEM.

uptake by the adipose tissues of the steroid-treated and control animals. However, the DOG uptake was slightly higher in hemidiaphragms of control than of steroid-treated animals ($P < 0.10$).

Discussion. Although oral glucose tolerance was impaired after 10 wk of steroid treatment, the serum insulin response to glucose was unaffected in our experiment. Prolonged treatment with oral contraceptives appear to cause hyperglycemia and hyperinsulinism in women (2, 11), even though, in some cases, glucose tolerance and insulin response had returned toward normal 2 and 3 yr after continued therapy (11). It is possible that a similar adjustment may have occurred in our rats after only 10 wk of steroid treatment.

No significant differences were observed in the insulin effect on serum glucose between steroid-treated and control rats. However, Beck (12) observed that rhesus monkeys, treated with large doses of progesterone for 3 wk, exhibited a much slower glucose disappearance rate after exogenous insulin administrations. This effect of progesterone could be overcome by increasing the dose of insulin. Thus, Beck (12) postulated that progesterone produced a mild, but significant peripheral resistance to the hypoglycemic effect of insulin.

The lipogenic effect of exogenous insulin, measured by the conversion of blood glucose into fatty acids by the adipose tissue was significantly depressed in the steroid-treated rats (Table I). A similar trend was observed for the conversion of blood glucose into adipose glyceride–glycerol, even though the results were nonsignificant. A similar conclusion had been reached as a result of experiments *in vitro* (6). Thus, the adipose tissue of steroid-treated rats appears to be resistant to the lipogenic effect of exogenous insulin both *in vivo* and *in vitro*, suggesting that this tissue may play a significant role in the observed alteration of carbohydrate and lipid metabolism.

DOG, which enters muscle tissue by an insulin-sensitive facilitated transport system, is not metabolized beyond its initial phosphorylation to 2-deoxyglucose-6-phosphate (13). This property made it suitable for measuring the effect of the steroids on glucose transport. As expected, DOG uptake by hemidiaphragm and adipose tissue increased with increasing concentrations of DOG in the medium. Although this uptake was slightly lower in the tissues of steroid-treated than in those of control rats, the differences were small and reached statistical significance only for the diaphragm. Thus, a decrease in glucose transport can account, at best, for only a small part of the insulin resistance observed in *in vitro* experiments (6). So it seems that this phenomenon most likely occurs beyond the point of glucose entry and its initial phosphorylation. Indeed, recent evidence indicates that contraceptive

steroids modify the activities of several enzymes of carbohydrate metabolism in human endometrium homogenates (14).

Summary. Oral contraceptive steroids, norethynodrel and mestranol, were fed to 11-wk-old female Sprague–Dawley rats, in combination and in quantities proportional to those used by women for contraceptive purposes. Three experiments were performed. The first experiment, demonstrated that 10 wk of treatment, impaired the animal's glucose tolerance, but not its insulin response to glucose. The second experiment demonstrated that 6 wk of steroid feeding, decreased the *in vivo* conversion of blood U-^{14}C-labeled glucose into adipose tissue fatty acids and into diaphragm glycogen, although the effect on the diaphragm was not statistically significant. In the third experiment, it was found that the uptake of 2-deoxyglucose-1-^{14}C by the adipose tissue removed from rats after 6 wk of treatment, was not different from that of control tissue, but the uptake by the hemidiaphragms was slightly lower.

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