

## Effects of Estrogen on Glucose Uptake by Rat Muscle<sup>1</sup>

HARRY SHAMOON<sup>2</sup> AND PHILIP FELIG<sup>3</sup>

*Department of Internal Medicine, Yale University School of Medicine,  
New Haven, Connecticut 06510*

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The isolated rat diaphragm was used to study the effects of 17 $\beta$ -estradiol on basal and insulin-mediated glucose uptake. Rats were injected with estradiol for 2 wk in daily doses of 10  $\mu$ g/100 g of body weight and were compared to untreated control animals. Estrogen treatment resulted in a 16% decrease in basal glucose uptake by diaphragm muscle as compared to controls. In contrast, in the presence of insulin, glucose uptake by muscle increased 103% above basal in estradiol-treated animals as compared to a 38% rise in the control group. The absolute rate of glucose uptake induced by insulin in the estradiol treated animals (5.8 mg/g/hr) was 22% higher than in controls. These findings were not accompanied by changes in weight gain, plasma glucose and plasma immunoreactive insulin concentrations in the treated animals. *In vitro* incubation of diaphragm muscle with estradiol did not have an effect on basal or insulin-mediated glucose uptake.

The data indicate that treatment with naturally occurring estrogens increases muscle sensitivity to insulin-stimulated glucose uptake. These findings suggest that the carbohydrate intolerance associated with the administration of oral contraceptives may be related to the use of synthetic rather than natural estrogens and/or progestins in such preparations.

### INTRODUCTION

Early attempts at understanding the interaction of sex steroids and carbohydrate metabolism showed that crude estrogenic preparations decreased glycosuria in diabetic animals (1). Subsequent reports were contradictory, but it was clear that the effects of estrogens on carbohydrate metabolism varied in relation to the animal model employed, the steroid preparation administered, and the doses used (2). Our interest in this subject was prompted by the observation that the administration

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of oral contraceptive agents is associated with a deterioration in glucose tolerance in 20–70% of women taking these agents (2, 3). Whether the diabetogenic effects of oral contraceptives are a consequence of the presence of synthetic rather than natural estrogens or are related to their progestin content has not been established (4). The present investigation was consequently carried out to determine if estrogen, specifically the naturally occurring  $17\beta$ -estradiol, affects basal and insulin-stimulated glucose uptake by muscle cells. In this study, the isolated rat diaphragm technique (5) was employed to evaluate the interaction of estrogen and insulin on glucose uptake by muscle.

## MATERIALS AND METHODS

Non-pregnant, adult, female rats of the Wistar strain (Camm Research, Wayne, NJ) were used in all the experiments. Animals were kept in individual cages for at least 10 days prior to the experiment. Food and water were allowed ad lib., the diet consisting of Purina Laboratory Chow. Room temperature was kept at 75–80°F with lighting from 6:00 A.M. until 6:00 P.M. Weight gain ranged from 15–25 g/wk; animals weighed 120–180 g at the time of study. In order to reduce diurnal variations, all rats were sacrificed between 8:00 and 10:00 A.M. on the day of the study.

Animals were fasted for 24 hr prior to the morning of the experiment but were allowed access to water. Rats were decapitated using a guillotine (Harvard Apparatus) following which the diaphragm was removed (6). After excision, the tissue was placed in 5 ml of iced Krebs-Ringer bicarbonate buffer (pH 7.4) saturated with 95%  $O_2$ /5%  $CO_2$ . The muscle was then trimmed and divided into two or four equal portions yielding hemi- and quarterdiaphragms, respectively. The latter were then preincubated at room temperature for 10 min in 10 ml of buffer containing glucose (150 mg/100 ml) to allow equilibration of the muscle (7). At the end of preincubation, each piece of muscle was transferred to a 10cc Erlenmeyer flask containing 1.0 ml of buffer to which glucose was added in a concentration of 150 mg/100 ml and 1% bovine serum albumin (BSA). The effect of insulin was studied by adding insulin in a concentration of 100  $\mu$ U/ml to some of the flasks. The flasks were gassed with 95%  $O_2$ /5%  $CO_2$ , sealed, and incubated at 37°C in a Dubnoff metabolic shaking bath at 100 oscillations/min for 60 min.

Glucose concentrations were measured initially and at the end of the incubation period in duplicate. Each piece of diaphragm was weighed at the end of incubation and glucose uptake could thus be expressed as milligrams of glucose per gram wet muscle per hour (mg/g/hr).

Since blood was not obtainable from decapitated rats, samples for glucose and immunoreactive insulin (IRI) levels were obtained in 10 animals by vena caval puncture under ether anesthesia.

Two groups of animals were studied to determine the effect of *in vivo* administration of estrogen on glucose uptake. Group I consisted of control animals injected subcutaneously with sesame oil (0.08–0.12 ml) daily for 2 wk. Group II rats were treated, with  $17\beta$ -estradiol in a dose of 10  $\mu$ g per 100 g body weight per day in a similar fashion as controls. This dose was chosen to achieve circulating estrogen concentrations sufficient to inhibit ovulation (8). For each animal in both groups, one hemidiaphragm was incubated with insulin and the other without insulin.

Additional studies were conducted on a separate group of uninjected animals to determine the effects of *in vitro* addition of estrogen on glucose uptake. Dia-

phragms were divided into four equal portions. In each animal, one quarter-diaphragm was incubated in a control flask containing glucose (150 mg/100 ml) and BSA (1%) in buffer, and the others were incubated with insulin (100  $\mu$ units/ml) and/or estradiol. The concentration of estradiol in the flasks was 500 pg/ml ( $2 \times 10^{-3}$   $\mu$ M) which is in excess of the peak plasma concentrations of 30 pg/ml observed in adult female Wistar rats (9).

In an additional set of experiments, quarterdiaphragms were pre-incubated with estradiol for 10 min at a concentration of 2  $\mu$ moles/liter and then added to flasks as described above.

The use of quarterdiaphragms allowed each animal to act as its own control, thus facilitating the statistical analysis as well as eliminating interanimal variability.

Insulin used was crystalline zinc insulin (Lilly, U100 Regular Insulin, Lot #2L13854) diluted in veronal buffer (barbital/Na diethylbarbiturate 0.02 M) containing 2% BSA. 17 $\beta$ -estradiol (estra-<sup>1,3,5(10)</sup>-triene-3,17-diol) was A Grade chromatographically pure steroid (California Biochemical Corp., Lot #201120). Dilutions were carried out in reagent grade ethanol followed by serial dilution in distilled water.

Glucose determinations were carried out by the glucose oxidase method (10) using the Glucostat Reagent Kit (Worthington Biochemical Corp., Freehold, NJ) with modifications (11).

Due to the large numbers of comparisons, a simple *t* test for significance was not appropriate. Hence, all experiments were initially analyzed according to a repeated measures analysis of variance design (12). Where an omnibus *F* test in the Anova proved to be significant, differences between means of individual groups were tested for significance using the Newman-Keuls procedure for evaluating *q*, the studentized range statistic, where  $q = (x_2 - x_1)/(s.e.)_x$ .

## RESULTS

### *In Vivo Studies*

The effects of estradiol administration on glucose uptake by rat hemidiaphragms is shown in Table 1. Estrogen treatment resulted in a 16% decrease in basal glucose uptake as compared to control ( $P < 0.001$ ). In both groups insulin resulted in a significant increase in glucose uptake above basal ( $P < 0.001$ ). However, the absolute rate of glucose uptake in the presence of insulin in the group receiving estrogen was 22% higher than in controls ( $P < 0.001$ ). This effect of estrogen on insulin-stimulated glucose uptake is even more evident when the change in glucose uptake as compared to the basal state is examined. The increment in glucose uptake induced by insulin in control animals was  $1.33 \pm 0.06$  mg/g/hr

TABLE 1  
EFFECT OF ESTRADIOL TREATMENT ON GLUCOSE UPTAKE BY HEMIDIAPHRAGMS  
(MEAN  $\pm$  SEM)

	N	Glucose uptake (mg/g/hr)		P
		Control	Estradiol	
Basal (no insulin)	19	3.49 $\pm$ 0.06	2.91 $\pm$ 0.06	<0.001
Insulin (100 $\mu$ m units/ml)	19	4.82 $\pm$ 0.06	5.88 $\pm$ 0.06	<0.001
P		<0.001	<0.001	

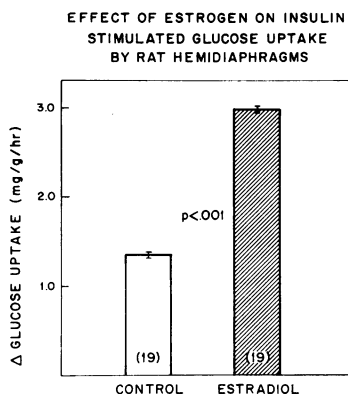


FIG. 1. Effect of estrogen (estradiol) on insulin-stimulated glucose uptake by rat diaphragm. The height of the bars represents the mean ( $\pm$ SEM) change in glucose uptake above basal levels induced by insulin in control (open bar) and estradiol-treated (hatched bar) animals.

(mean  $\pm$ SEM) whereas in estradiol-treated rats the increment was  $2.97 \pm 0.06$  mg/g/hr ( $P < 0.001$ ) (Fig. 1).

These changes representing an increase in uptake of 38% above basal in the control animals as compared to a 103% increase above basal levels in estradiol-treated animals.

In Table 2 the body weights and concentrations of plasma glucose and insulin are shown. There were no significant differences with respect to body weights, plasma glucose or immunoreactive insulin concentrations after 2 wk of treatment with estrogen as compared to controls.

#### *In Vitro Studies*

In Table 3, the effect of *in vitro* addition of estradiol to rat quarterdiaphragms is shown. In the initial set of pilot experiments (Expt A) insulin addition was found to increase glucose uptake from  $2.65 \pm 0.22$  mg/g/hr in the basal state to  $4.33 \pm 0.38$  mg/g/hr ( $P < 0.001$ ). The addition of estradiol ( $2 \times 10^{-3}$   $\mu$ M) *in vitro* had no effect on basal glucose uptake (Expt B). In Expt C, the *in vitro* addition of estradiol is shown to have no effect on insulin-stimulated uptake of glucose. In the final group of rats (Expt D), quarterdiaphragms from each animal were preincubated at 2  $\mu$ M concentrations of estradiol prior to the usual incubation. Glucose uptake with insulin treatment was again significantly greater than

TABLE 2  
FASTING PLASMA GLUCOSE, IMMUNOREACTIVE INSULIN (IRI) AND BODY WEIGHTS IN  
CONTROL AND ESTRADIOL-TREATED RATS (MEAN  $\pm$  SEM)

	N	Glucose (mg/100 ml)	IRI ( $\mu$ m units/ml)	Weight (g)
Control	5	99.2 $\pm$ 3.7	34.0 $\pm$ 2.8	155 $\pm$ 8.3
Estradiol-treated	5	97.6 $\pm$ 3.9	33.8 $\pm$ 2.9	158 $\pm$ 5.2
Significance (P)*		—	—	—

\* By Student's *t* test.

TABLE 3  
GLUCOSE UPTAKE (MEAN  $\pm$  SEM) BY QUARTERDIAPHRAGMS TREATED UNDER  
VARYING CONDITIONS IN VITRO

	N	Treatment condition			
		Control	Insulin	Estradiol	Insulin and estradiol <sup>c</sup>
Expt A	10	2.65 $\pm$ 0.33	<sup>a</sup> 4.33 $\pm$ 0.38		
Expt B	4	3.59 $\pm$ 0.38		3.53 $\pm$ 0.29	
Expt C	23	3.74 $\pm$ 0.22	<sup>b</sup> 5.25 $\pm$ 0.27		<sup>b</sup> 5.28 $\pm$ 0.26
<sup>c</sup> Expt D	9	3.22 $\pm$ 0.26	<sup>b</sup> 4.66 $\pm$ 0.19		<sup>b</sup> 4.60 $\pm$ 0.28

<sup>a</sup> Significantly different from control ( $P < 0.01$ ).

<sup>b</sup> Significantly different from control ( $P < 0.05$ ).

<sup>c</sup> In Expt D, all quarterdiaphragms were preincubated with estrogen for 10 min in a concentration of 2  $\mu$ moles/liter.

control (4.66  $\pm$  0.19 vs 3.22  $\pm$  0.26 mg/g/hr), but preincubation followed by incubation with estradiol and insulin was associated with no change as compared to insulin alone.

Since the estradiol was dissolved in ethanol, the effect of ethanol addition (0.1% by volume) on insulin-stimulated glucose uptake was also examined in 10 rats. Glucose uptake in control flasks was 4.16  $\pm$  0.31 mg/g/hr; in flasks containing insulin the uptake was 5.79  $\pm$  0.36 mg/g/hr ( $P < 0.05$ ) and in flasks containing insulin plus ethanol the uptake was 5.69  $\pm$  0.40 mg/g/hr. This represented no significant difference from insulin treatment alone.

## DISCUSSION

The discovery that anterior pituitary extract was diabetogenic in the rat and that hypophysectomy ameliorated the diabetic state (13) prompted studies of the effects of estrogen on carbohydrate metabolism. Crude estrogenic preparations were found to decrease glycosuria in diabetic animals (1, 14), yet could also exacerbate diabetes (15, 16). Houssay and his associates showed that the incidence of diabetes following subtotal (95%) pancreatectomy in male rats was 100% in 6 mo but only 20% in female rats (16). The "protective" effect of the female sex hormones was also suggested by studies in which castration followed pancreatectomy (17) and by the demonstration that exogenously administered estrogens reduced the incidence of diabetes after pancreatectomy, especially in the male (18).

In humans evidence regarding the effect of sex steroids on carbohydrate metabolism has been accumulated since the advent of oral contraceptive agents. In women treated with a synthetic estrogen-progestin combination, as many as 75% developed abnormalities in oral glucose tolerance testing (19). Subsequent studies suggested that the agent responsible for this deterioration in carbohydrate tolerance was the estrogenic component (2, 3).

Our studies demonstrate that short-term administration of the naturally-occurring 17 $\beta$ -estradiol in doses approximating those in oral contraceptives, results in significantly greater insulin-mediated uptake of glucose by skeletal muscle. These findings are thus compatible with previous data demonstrating that estrogens have a protective effect on the development of diabetes. However, the mechanism postu-

lated by the earlier studies, namely that estrogens increased endogenous insulin secretion (13, 17, 18) is not borne out by our findings (Table 2). In our animals, there were no changes in immunoreactive insulin concentrations, indicating that hyperinsulinemia is not a consequence of estrogen treatment nor a prerequisite for improved glucose uptake by peripheral tissue. The current data suggest that estrogens increase the sensitivity of skeletal muscle to insulin-stimulated glucose uptake. This enhanced response can account for improvement of the diabetic state in partially pancreatectomized animals since small amounts of endogenous insulin are likely to be secreted by the pancreatic remnant.

The current data challenge the notion that all estrogens are diabetogenic (2, 3). It should be noted, however, that recent studies indicate that only synthetic estrogens, particularly when combined with 19-nortestosterone progestins, cause a deterioration in glucose tolerance (4). In contrast, natural estrogens fail to demonstrate this effect (20). Our data support the validity of these conclusions in that the naturally-occurring estrogen,  $17\beta$ -estradiol, was found to increase rather than decrease insulin sensitivity in muscle. In addition, the data suggest that the impairment of carbohydrate tolerance with pregnancy cannot be ascribed to estrogen but is probably a consequence of other gestational hormones.

In order to determine if the effects of *in vivo* administration of estrogen involved a direct hormonal interaction at the tissue level, *in vitro* incubations with estradiol plus insulin were conducted. We found that even at supranormal concentrations of  $17\beta$ -estradiol, there were no changes in basal or insulin-mediated glucose uptake. In addition, there was no evidence that other factors (i.e., ethanol diluent) could be interfering. Preincubation with estradiol demonstrated that even with periods exceeding 10 min there was no direct effect of estrogen on skeletal muscle uptake of glucose. This insensitivity of muscle to incubation with estrogens may reflect the fact that under physiologic circumstances rat diaphragm muscle fails to accumulate estrogens in a manner comparable to uterine muscle (21).

As contrasted to the action of insulin, we found that estrogen treatment was associated with a 16% decrease in basal glucose uptake as compared to control. The failure of this change in glucose uptake to be reflected in alterations in the fasting plasma glucose concentrations is explainable when one considers the relative contribution of resting muscle to total systemic glucose turnover. In the postabsorptive state the vast bulk of circulating glucose is taken up by non-insulin-dependent tissues (brain, nerve, blood cells) with muscle depending largely on fatty acids and ketone bodies as sources of energy. Even when glucose, insulin and fatty acids are present in greater concentrations (e.g., after a meal), glucose accounts for as little as one-fifth of the oxidative metabolism of resting muscle (22). Thus, a relatively large change in uptake of glucose by resting muscle in the fasted state would not perceptibly affect blood glucose levels.

It is noteworthy that while estrogen had opposing effects on basal and insulin-stimulated glucose uptake, these actions may be interrelated. In the intact animal, a two- to threefold increase in the sensitivity of diaphragm muscle to insulin would result in an increased pool of intracellular glycogen (by increasing availability of glucose and/or induction of enzymes). When removed from the animal, muscle metabolism becomes wholly dependent on intracellular stores of energy. Thus muscle from estrogen-treated animals, having a higher content of glycogen, would be expected to have a relatively smaller need for exogenous glucose. A consequent decrease in basal glucose uptake may thus be explained.

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