Effect of physical training on insulin response in late pregnancy rats

Efecto del ejercicio físico sobre la respuesta insulínica en ratas gestantes a término

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ABSTRACT

Oral glucose tolerance test (2 g/Kg body weigth) produced parallel changes in blood glucose in both 20 day pregnant and virgin rats whereas plasma RIA -insulin increased more in the pregnant animals indicating insulin resistance. This study was addressed to determine whether a moderate maternal aerobic exercise throughout gestation modifies insulin responsiveness in the mother. Virgin and pregnant rats subjected or not to a treadmill running 10° grade for 5 days per week at 20 m/min until 75 min on 20 day of protocol and/or gestation were subjected to an intravenous tolerance test with 10 IU of porcine insulin/Kg body weight. The integrated hypoglycemic effect of intravenous insulin administration showed an enhanced insulin responsiveness in the exercised pregnant rats as compared to non-exercised animals, with no effect in virgin rats.
Key words: Pregnancy. Rat. Glucose and insulin tolerance. Exercise. Endocrine pancreas.

RESUMEN

Una dosis oral de glucosa (2 g/Kg de peso) produjo cambios similares en la glucosa sanguínea de ratas gestantes de 20 días y vírgenes mientras que el aumento en la insulina plasmática fue mayor en los animales gestantes, indicando resistencia a la insulina. En el presente trabajo se estudió si un ejercicio moderado y aerobio durante la gestación modifica la respuesta a la insulina en la madre. Ratas vírgenes y preñadas de 20 días corrieron en una cinta rodante inclinada 10° durante 5 días/semana a 20 m/min e incremento progresivo hasta 75 min en el día 20 de ejercicio y/o gestación en que fueron sometidas a un test de tolerancia intravenoso de insulina con 10 IU de insulina porcina/Kg peso. El efecto hipoglucemiante de la insulina intravenosa mostró una mayor respuesta a la insulina en las ratas preñadas ejercitadas con respecto a las no ejercitadas, mientras que no se observó efecto en las ratas vírgenes.
Palabras clave: Gestación. Rata. Tolerancia a glucosa e insulina. Ejercicio. Páncreas endocrino.

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INTRODUCTION

It is well known that during late gestation in both human and experimental animals there is a decline of blood glucose concentration in the post-absortive period or after short periods of starvation (1,2,3,4) although blood glucose concentrations rapidly return to normal values after parturition. In spite of this tendency toward hypoglycemia, plasma insulin levels are elevated in late pregnancy (2,5,6).

Glucose tolerance at late pregnancy remains within normal nonpregnant limits (1,7) despite the increased insulin secretion rate in response to glucose (8,9), indicating a state of insulin resistance. Decreased insulin sensitivity has been well documented in late pregnancy in both women (10,11) and rats (8,12,13) and the primary site of insulin resistance during gestation is skeletal muscle (3,14).

In contrast to pregnancy, exercise in the nonpregnant state increases insulin sensitivity (15) and improves glucose tolerance in both humans (16,17) and rats (18).

Thus, the present study was designed to investigate insulin responsiveness in the late-pregnant and virgin control rats subjected to aerobic exercise protocol.

MATERIAL AND METHODS

Animals and Experimental Desing

Female Wistar rats from our own colony (Center for Animal Experimentation 86/609/UE; reference number 2800J-22A) were used. They were mated when weighing 160-180 g, and gestation was timed by the appearance of spermatozoa in vaginal smears. Animals were housed in individual cages in a light-and temperature controlled room (12h on-off cycle, 22+2 °C). They had free access to water and chow pellets (Panlab. Barcelona, Spain). Rats were always studied under fed conditions on day 20 of pregnancy, and age-and sex-matched virgin rats were always studied in parallel.

Exercise Protocol

It consisted of a treadmill run 10° grade for 5 days per week starting at

the speed of 20 $m.min^{-1}$ during 20 min and a progressive daily time increase attaining 75 min on the day 20 of experiment or pregnancy.

To evaluate lactate concentration, blood samples collected from the tip of the tail were inmediately deproteinized with frost perchloric acid (0.165 mol/l) and analyzed by a lactate deshydrogenase method (Boehringer-Mannheim).

Glucose Tolerance Tests

Virgin and 20 days pregnant rats were subjected to an oral glucose tolerance test (OGTT) as previously described (19) and blood glucose concentration was measured at 0 (before glucose load), 7.5, 15, 22.5, 30, 60 and 120 min (after glucose load) with a Reflolux II analyzer (B/Test/Glycemie 20-800 R. Boehringer-Mannheim). Plasma aliquots were kept at -80 °C until analysed for RIA-insulin with a rat specific radioinmunoassay kit (20).

Content of Insulin in the Pancreas

One set of virgin and 20 days pregnant rats was decapitated for dissection of the pancreas, which was rapidly placed in ice-cold Hanks' solution; any fat tissue was eliminated under a magnifier. After weighing, the insulin was extracted in acid ethanol (21). The extract was neutralized with saturated sodium bicarbonate for insulin assay (20).

Intravenous Insulin Tolerance Test

Another set of rats, untrained and trained animals, was subjected to an intravenous insulin tolerance test (IITT) as previously described (22). Blood was collected from the tip of the tail at 0 (before insulin or saline administration), 4 and 8 min after the insulin or saline injection, and glucose concentration was measured as above. Each time point after insulin injection was corrected by values found at the same time period in animals receiving saline. The insulin effect was expressed as the area under the corrected insulin effect curve (22).

Expresion of the Results

Results are expressed as means ± SEM. Statistical comparisons between groups were carried out by using the Student's "t" test for two groups and by

the Student-Newman-Keuls test for more than two groups by using the "Graph Pad Instat" of IBM and considering significant differences between the groups when $p<0.05$.

RESULTS

The change in blood glucose at different times after the glucose load is shown in figure 1. Blood glucose in 20 day pregnant rats did not differ from those of virgin animals except a faster decline at 120 min in the former. In spite of this similarity in blood glucose values, insulin levels reached in the 20 day pregnant rats were significantly higher than in virgin controls shortly after the glucose load (7.5 and 15 min) whereas at later test times they declined more rapidly (fig. 2).

As shown in table 1, the weight of the pancreas was greater in pregnant than in control rats and both the pancreatic insulin concentration and the total pancreas insulin content appeared augmented on day 20 of gestation as compaired to virgin control animals.

Exercise protocol was addressed in another set of rats to evaluate insulin responsiveness in late-pregnant and virgin control rats. Training did not modify food intake or body weight free of conceptus of the rats, neither conceptus weight nor placentas or fetuses weight, nor number per litter (data not shown). As shown in table 2, blood lactic acid levels were measured under basal conditions in the four groups, and at 15, 35, 55, 65 and 75 min of the treadmill run in both exercised pregnant and virgin rats. With the exception of a significant decrease in lactic acid levels found in blood of 20 day pregnant control, no value in any of the remaining groups rats differed among groups, values prior to the exercise protocol being the same as those found just after its completion (table 2).

Blood glucose and insulin levels at day 20 of the exercise and/or pregnancy are shown in table 3. Glucose levels are lower in pregnant than in virgin rats and the exercise protocol did not modify these levels in both exercised

Table 1.—weight and insulin on the pancreas

	Weight (g)	*Insulin concentration (μU/g tissue)*	*Total insulin content (μU/pancreas)*
0 days	0.79±0.01	73.96±0.96	58.32±0.84
20 days	0.99±0.01[a]	522.37±8.24[a]	518.41±7.91[a]

Data are mean ±SEM of 5-33 rats/group.
a, $p<0.001$ vs. values in virgin rats (day o).

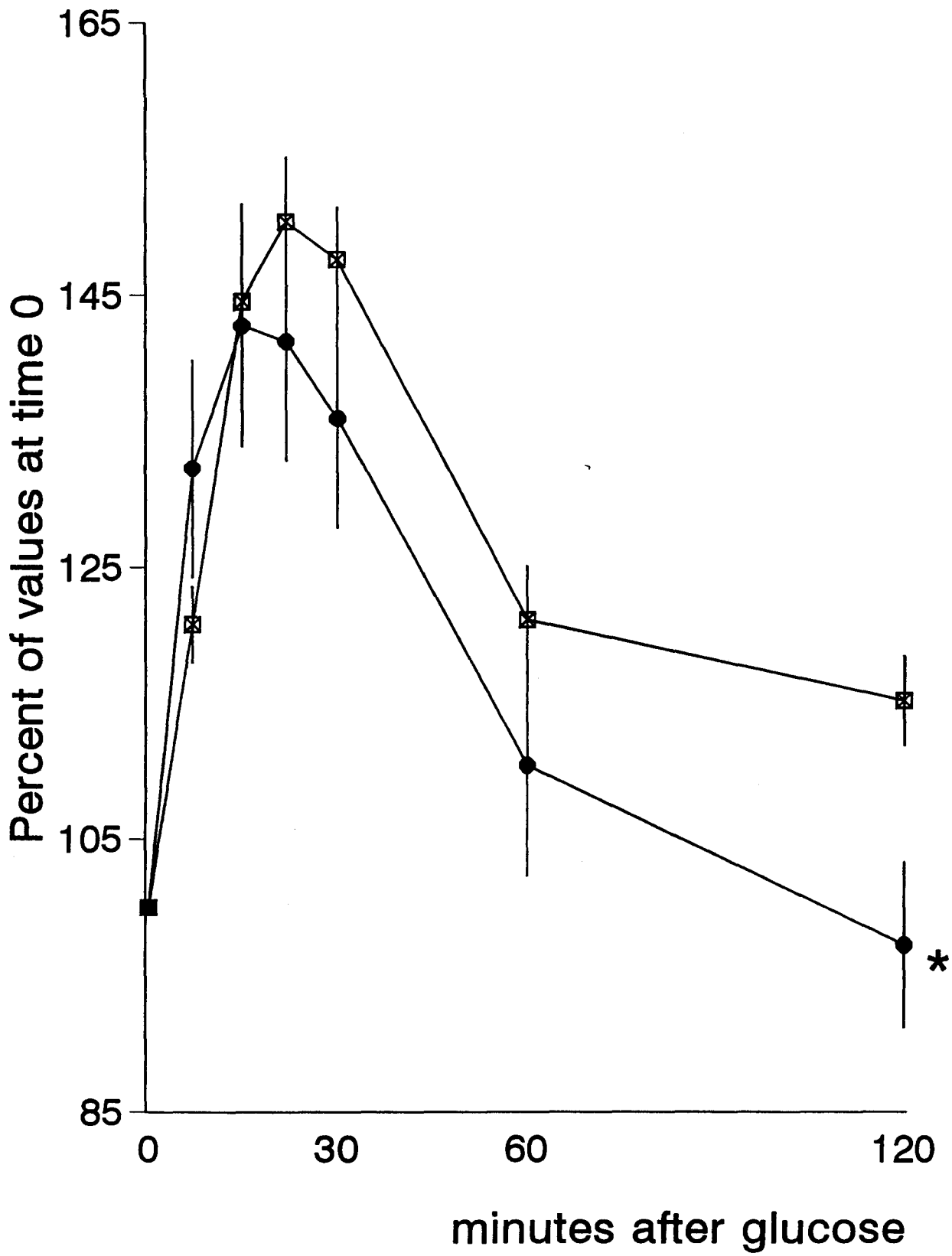

Fig. 1.—Percent changes in circulating glucose levels after 2g of oral glucose/Kg body weight at different times to virgin (white square) and 20 day pregnant rats (black circle). Data are means ± SEM of 8-33 rats/group. *$p<0.05$, vs. values in virgin rats.

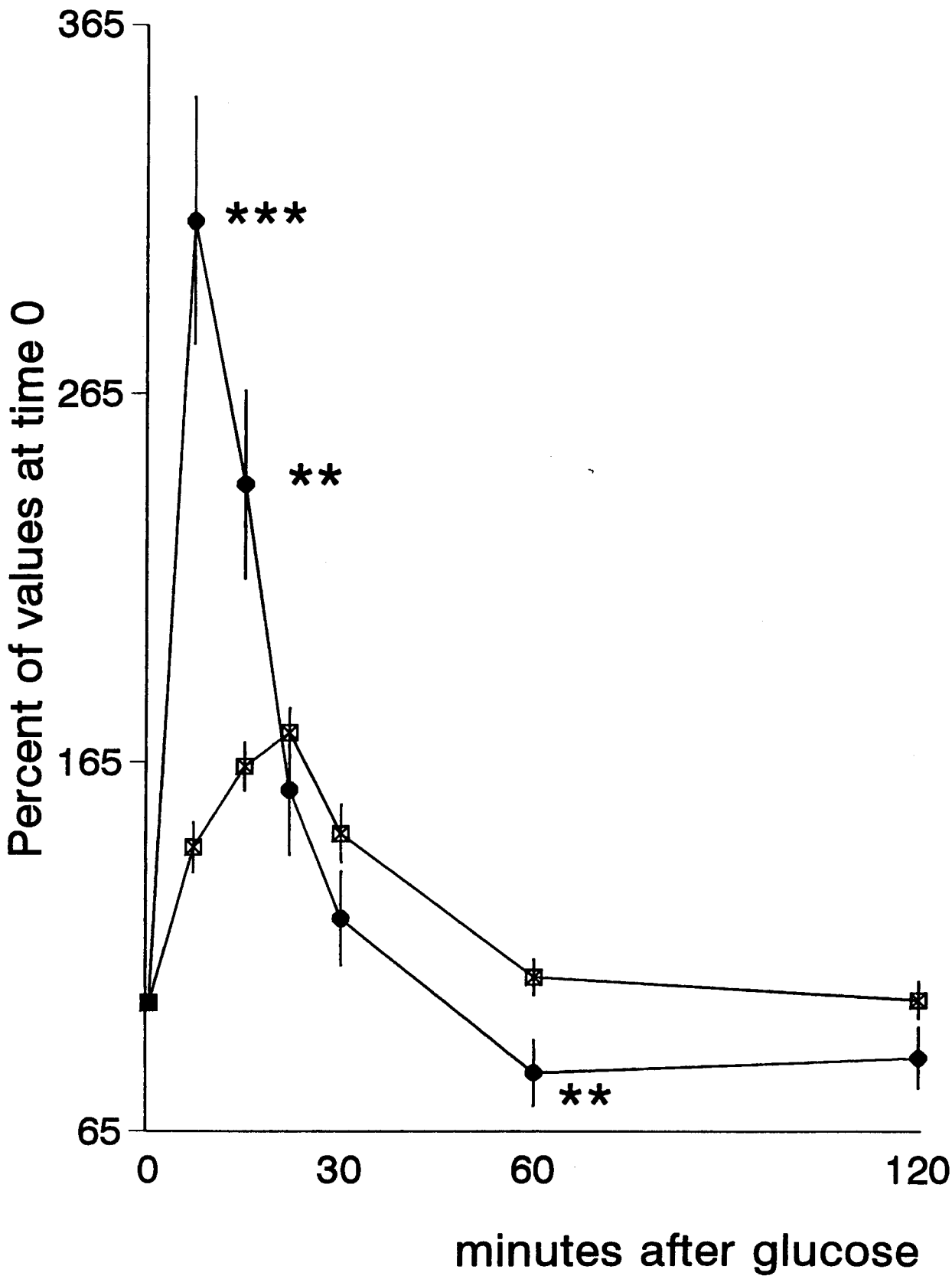

Fig. 2.—Percent changes in circulating insulin levels after 2g of oral glucose/Kg body weight at different times to virgin (white square) and 20 day pregnant rats (black circle). Data are means ± SEM of 8-33 rats/group. **p<0.01, ***p<0.001, vs. values in virgin rats.

Table 2.—lactate concentration at times of trainig

	Lactate concentrations (mmol/l)		
Control virgin (basal)	1.280±0.13 (basal)	Control pregnant	3.125±0.62
Exercised virgin (basal)	1.233±0.38	Exercised pregnant (basal)	0.902±0.19 [a]
Time of training (min)		Time of training (min)	
t:15	1.607±0.30	t:15	1.696±0.31
t:35	1.540±0.23	t:35	1.540±0.16
t:55	1.106±0.19	t:55	1.304±0.09
t:65	1.120±0.08	t:65	1.635±0.34
t:75	1.210±0.24	t:75	1.413±0.17

Data are mean ± SEM of 8 rats/group.
a, $p<0.05$ vs. values in control pregnant rats.

Table 3.—blood glucose and plasma insulin levels in the exercised an unexercised groups.

	Glucose (mg/dl)	*Insulin (μU/ml)*
Control virgin	106.5±5.2	62.7±0.9
Exercised virgin	123.8±7.3	56.2±5.3
Control pregnant	78.6±3.7[a]	96.5±6.6[a]
Exercised pregnant	79.5±3.0[b]	117.8±14.7[b]

Data are means ± SEM of 6-14 rats/group.
a, $p<0.001$ vs. values in control virgin rats.
b, $p<0.001$ vs. values in exercised virgin rats.

groups. Plasma insulin levels are higher in pregnant than in virgin rats. The exercise protocol slightly increased plasma insulin levels in exercised pregnant rats.

To obtain an index of insulin responsiveness in both untrained and trained animals, both virgin and 20 day pregnant rats received a high intravenous dose of insulin (10IU/Kg body weigth) or saline, and its corrected effect on blood glucose was measured during the following 8 min. As shown in figure 3, the integrated hypoglycemic effect of insulin was intensely and significantly decreased in control pregnant as compared with control virgin rats. The exercise protocol did not modify this effect in trained virgin animals when compaired to unexercised virgin rats, but significantly increased the area in trained pregnant rats as compaired with control pregnant.

DISCUSSION

Using OGTT in 20 day pregnant rats, present results in concur with previous findings indicating that insulin resistance becomes clearly manifested

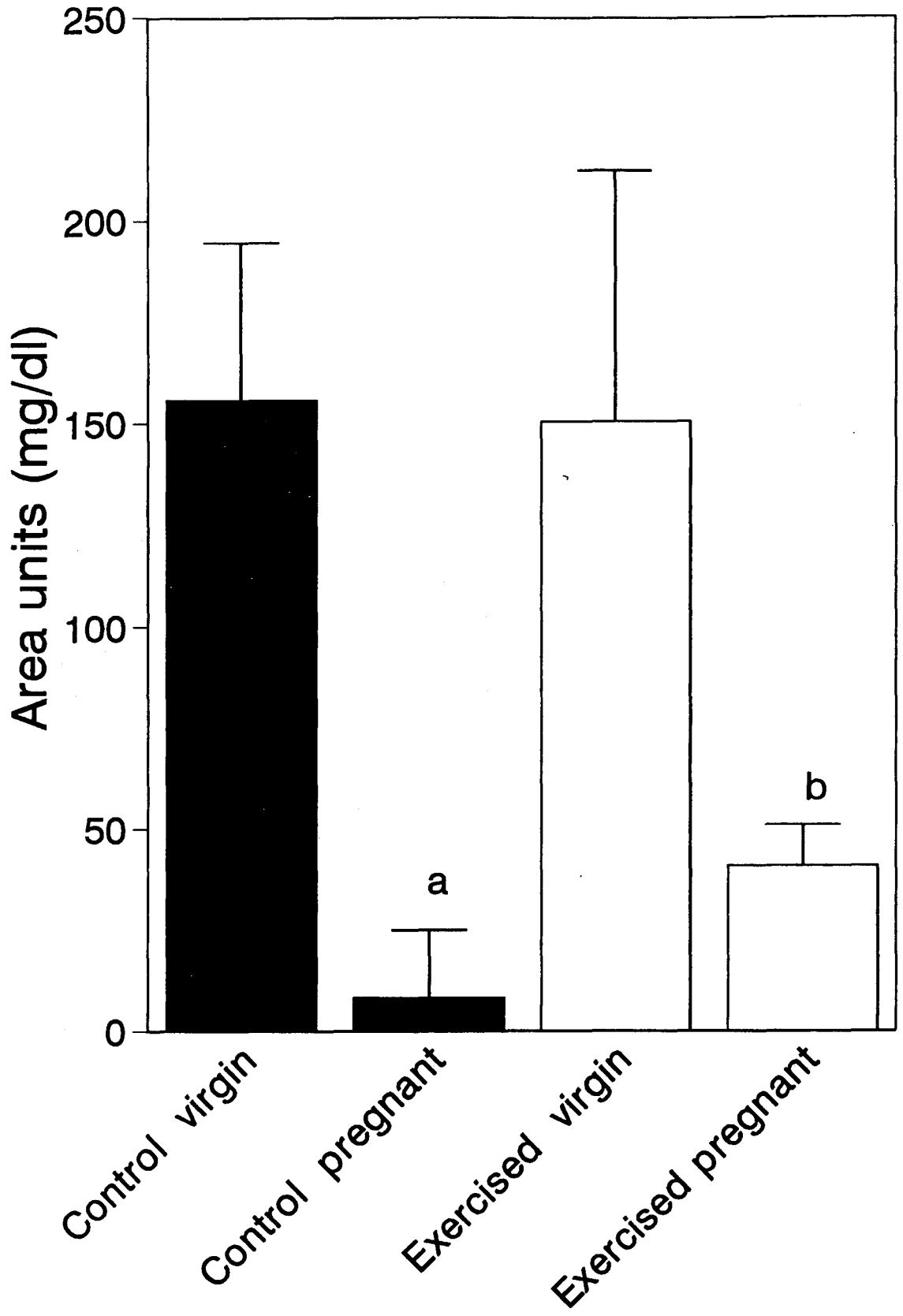

Fig. 3.—Corrected hypoglycemic effect of intravenous insulin (10IU/Kg body weight) on control groups (black bars), and exercised groups (white bars). Data are means ± SEM of 6-14 rats/group. a, $p<0.001$ vs. values in control virgin and b, $p<0.05$ vs. values in control pregnant.

during late gestation (8,12,14,22). At this late phase of gestation we have shown here that the pancreas of the mother rat accumulates an enormous amount of insulin and, besides maintaining elevated basal levels of insulin, whenever she eats she releases a greater amount of insulin into circulation, as indicated by the exaggerated increase in plasma insulin after oral glucose administration.

Previous findings in exercised rats also showed a differential metabolic effect between pregnant and nonpregnant rats (23,24), but our study shows that exercise protocol improves insulin responsiveness in pregnant rats but not in virgin rats. The training used here is aerobic as indicated by the unchanged blood lactate levels at different times of the treadmill run. An increment in blood lactate levels was, however, seen in the control 20 day pregnant rat as compared to virgin rats, but this is not surprising because it is known that lactic acid is produced by the placenta and the fetus (25,26,27,28,29). This increase in control pregnant does not appear in the exercised pregnant rat, probably due to of its enhanced use as gluconeogenic substrate during training (30,31). The situation of differential response between pregnant and virgin rats to an exercise protocol modifying insulin responsiveness seen here contrasts with the enhanced insulin responsiveness described in rats subjected to other exercise protocols (32,33). However, this study concurs with previous ones in that moderate exercise does not modify insulin responsiveness in nonpregnant rats (34). Pregnancy induces many changes in the mother, endocrine, metabolic and physiological adaptations which can modify her response to exercise (35). Thus, this differential situation may justify a different exercise responsiveness in nonpregnant and pregnant individuals .

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