Metabolism
Clinical and Experimental

VOL 48, NO 11 NOVEMBER 1999

Effect of Pregnancy on Insulin Metabolism in Spontaneously Hypertensive Rats

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Several lines of evidence suggest that insulin resistance and/or hyperinsulinemia may play an important role in the pathogenesis of hypertension. We studied the effect of pregnancy on insulin metabolism in spontaneously hypertensive rats (SHRs) and in Wistar-Kyoto rats (WKYs) as a control. Pregnancy markedly reduced blood pressure in both strains of rats, but insulin resistance as determined by the hyperinsulinemic glucose clamp (10 mU/kg/min) increased in SHRs and was unchanged in WKYs. The plasma insulin response to an intravenous glucose challenge in SHRs was low and did not change with pregnancy. Therefore, it is suggested that the regulation of blood pressure in these animals is linked to an unknown factor rather than to insulin resistance and hyperinsulinemia. Fetuses from SHRs had a lower body weight and plasma glucose level and higher plasma insulin and pancreatic insulin levels than those from WKYs. Thus, fetal hyperinsulinemia in the SHR may be linked to the development of hypertension in adulthood.

IT IS WELL DOCUMENTED that insulin plays an important physiological role in regulating both the maternal and fetal fuel during pregnancy.[1] Pregnancy causes a decrease in plasma glucose and an increase in plasma insulin in rodents and humans. During late gestation, insulin resistance occurs, resulting in compensatory augmented insulin secretion.[2-4] The pathogenesis of essential hypertension remains to be fully investigated. It has been suggested that increased insulin resistance may be responsible for hypertension.[5,6] The spontaneously hypertensive rat (SHR) has been used as an animal model to assess the relationship between insulin resistance and hypertension. Previous studies[7,8] using the glucose-clamp method demonstrated impaired insulin sensitivity in SHRs compared with normotensive Wistar-Kyoto rats (WKYs), whereas Buchanan et al[9,10] argued against these findings and showed that the SHR was hyperinsulinemic without insulin resistance. To further clarify the relationship between hypertension and insulin resistance, the effect of pregnancy has been examined in both strains of rats.

A recent epidemiological study showed that growth retardation in fetal life was strongly related to high blood pressure in adult life,[11] but this finding has not been confirmed in experimental animals. Although a previous study[12] found that the body weight is lower in fetuses from SHRs versus WKYs as a control, there were no available data with regard to the relationship between insulin metabolism and fetal growth in these experimental animals. In the present study, we examined the effect of pregnancy on insulin metabolism in SHR mothers and fetuses.

MATERIALS AND METHODS

SHRs and WKYs were purchased from Charles River Laboratories (Atsugi, Japan). The animals were housed in air-conditioned quarters at 24°C under artificial lighting (8 AM to 8 PM). Tap water and chow pellets were provided ad libitum. Mating was accomplished by overnight housing of females with males and confirmed by the presence of sperm in a vaginal smear the next morning. Midnight on the night of mating was considered the start of day 0 of embryogenesis.

On day 18 of gestation, the euglycemic insulin clamp study was performed. All of the experiments were performed between 8 and 11 AM as described previously.[3] The rats were anesthetized with pentobarbital (30 mg/kg body weight intraperitoneally). To avoid hypothermia, the rats were placed on a heating blanket maintained at 37°C during the experiments. Polyethylene catheters (PE-50; Becton Dickinson, Mountain View, CA) were inserted into the jugular and femoral veins. Human insulin (Novolin R; Novo Nordisk, Copenhagen, Denmark) in 0.9% NaCl with 0.25% bovine serum albumin was then infused into the femoral vein at 10 mU/min/kg. Blood glucose, measured in samples from the tail vein, was kept at the basal level by determining the blood glucose every 5 to 10 minutes and empirically adjusting the infusion rate of a 20% glucose solution (Ootsuka, Tokushima, Japan). The rate of insulin-mediated glucose uptake (glucose infusion rate [GIR]) was thus determined by the steady-state blood glucose concentration during the euglycemic clamp. The GIR was assessed as the mean value from 50 to 80 minutes.

After the clamp study, the catheter was tunneled under the skin,

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Submitted March 21, 1995; accepted May 18, 1999.

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0026-0495/99/4811-0002$10.00/0

Table 1. Body Weight, Steady-State Blood Glucose, GIR, and Glucose Metabolic Clearance Rate in WKYs and SHRs

Group	No. of Rats	Body Weight (g)	SBG (mg/dL)	GIR (mg/min/kg)	MCR (mL/min/kg)
WKY					
Nonpregnant	6	245 ± 2	98 ± 1	20.6 ± 1.0	20.2 ± 0.4
Pregnant	8	294 ± 5	59 ± 1	18.8 ± 1.3	32.2 ± 2.6
SHR					
Nonpregnant	6	198 ± 5†	111 ± 4†	24.0 ± 1.0	21.8 ± 1.0
Pregnant	8	242 ± 3†	65 ± 1*	20.6 ± 1.1	31.4 ± 1.5

NOTE. Values are the mean ± SE.

Abbreviations: SBG, steady-state blood glucose; MCR, metabolic clearance rate.

*$P < .05$, †$P < .001$ *v* corresponding WKY.

exiting the animal through the back skin, and filled with polyvinylpyrrolidone-heparin. On day 20 of gestation, the food was removed at 8 AM and an intravenous glucose tolerance test (0.5 g/kg body weight) was performed without anesthesia. Blood samples were obtained immediately before and 3, 5, and 15 minutes after glucose administration.

Systolic blood pressure was measured by the tail-cuff method using an automatic blood pressure recorder (UR-1000; Ueda, Tokyo, Japan) after the rat was warmed to 37°C for 15 minutes.[13] All virgin rats were accustomed to the measurement of blood pressure before mating, but to avoid an abortion due to stress, the animals were not accustomed after conception. Blood pressure in pregnant rats was measured on 20 gestational days and the average of five readings was calculated. Then, the rats were killed by neck dislocation. Fetuses and placentas were removed by cesarean section, trimmed, and weighed. Blood samples in the fetuses were obtained from the axillary vessels. The plasma in the fetuses from each litter was pooled and frozen until assay.

The plasma glucose level was measured by the glucose oxidase method with a glucose analyzer (Fuji Film, Tokyo, Japan). Immunoreactive insulin was determined by specific radioimmunoassay[14] using rat insulin as a standard.

Results are presented as the mean ± SEM. Data were analyzed by ANOVA followed by Fisher's multiple-comparison test. When needed, Student's *t* test or the χ^2 test were used.

RESULTS

The body weight of both virgin and pregnant rats (18 gestational days) was significantly lower in SHRs versus WKYs (Table 1). Blood glucose was clamped at the level measured in the basal state, and was higher in SHRs versus WKYs in nonpregnant animals (105 ± 2 *v* 115 ± 3 mg/dL, $P < .05$). Pregnancy decreased plasma glucose, as shown by the steady-state blood glucose level. In contrast to previous reports in male rats,[7,8] the GIR was higher in female SHRs versus WKYs, although there was not a significant difference ($P = .058$). Pregnancy significantly ($P < .05$) decreased the GIR in SHRs but not in WKYs. Since glucose levels were decreased by pregnancy, the metabolic clearance rate was remarkably increased by pregnancy, although there was no significant difference between SHRs and WKYs.

Figure 1 illustrates the influence of pregnancy (20 gestational days) on pancreatic β-cell function in WKYs and SHRs. In nonpregnant rats, nonfasting plasma glucose levels were higher in SHRs versus WKYs (205 ± 13 *v* 174 ± 6 mg/dL), although there was no statistical significance. The plasma glucose response was greater in SHRs compared with WKYs, but a significant difference was obtained only at 15 minutes after glucose injection (388 ± 53 *v* 229 ± 10 mg/dL, $P < .05$). Pregnancy decreased the glucose response in both strains of

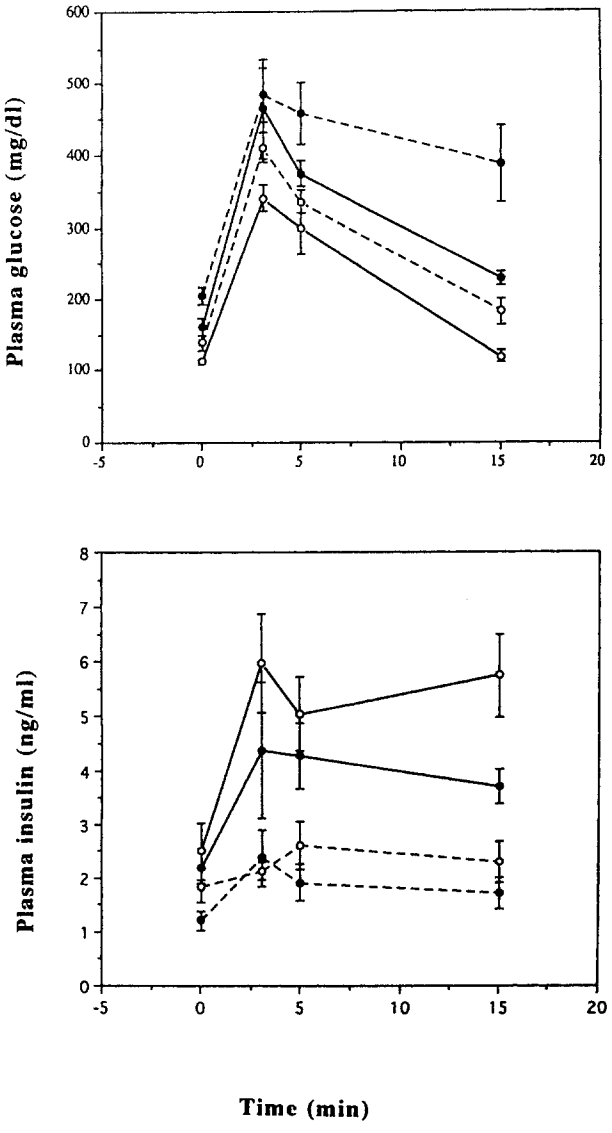

Fig 1. Plasma glucose and insulin responses to intravenous injection of glucose (0.5 g/kg body weight) in WKYs and SHRs. (-●-) Nonpregnant WKYs, (-○-) pregnant WKYs, (--●--) nonpregnant SHRs, (--○--) pregnant SHRs. Values are the mean ± SE of 4 to 6 observations.

Table 2. Pancreas Dry Weight, Insulin Content, and Insulin Content per Dry Weight in Nonpregnant and Pregnant SHRs and WKYs

Group	No. of Rats	Pancreas Dry Weight (mg)	Insulin	
			Content (μg)	Content/ Dry Weight (μg/mg)
Virgin				
WKY	9	273 ± 9	74.0 ± 4.8	0.28 ± 0.02
SHR	11	227 ± 7*	119.2 ± 4.7*	0.52 ± 0.02*
Pregnant				
WKY	5	270 ± 13	101.2 ± 9.3†	0.38 ± 0.04†
SHR	5	208 ± 13*	115.0 ± 5.7	0.56 ± 0.05*

NOTE. Values are the mean ± SE.
*$P < .001$ *v* corresponding WKYs.
†$P < .05$ *v* corresponding virgin rats.

rats. There was a significant difference in plasma glucose levels at 3 minutes after glucose injection between SHRs and WKYs (411 ± 21 *v* 313 ± 23 mg/dL, $P < .05$).

The plasma insulin response to glucose stimulation in nonpregnant SHRs was low and was significantly lower at all time points versus the response in WKYs. In addition, pregnancy had no effect on insulin secretory activity in pancreatic β cells in SHRs. On the other hand, pregnancy augmented β cell function in WKYs.

In virgin rats, blood pressure was higher in SHRs versus WKYs (196 ± 2 *v* 141 ± 3 mm Hg, $P < .001$). On day 20 of gestation, blood pressure was remarkably decreased in both SHRs (143 ± 5 mm Hg) and WKYs (118 ± 3 mm Hg). The decrement in blood pressure was much greater for SHRs versus WKYs. However, 4 or 5 days after delivery, blood pressure in SHRs was significantly ($P < .0001$) increased (180 ± 2 mm Hg).

The dry weight of the pancreas was lower for SHRs versus WKYs in nonpregnant animals (Table 2). There was no increase in the dry weight of the pancreas in pregnant rats. The insulin content of the pancreas was 1.6-fold greater in SHRs versus WKYs before pregnancy, although the body weight was 20% less in the former versus the latter (Table 1). There was a significant increase of the insulin content in pregnant WKYs, but no change was observed in pregnant SHRs. The insulin content per dry weight was greater in SHRs versus WKYs, and pregnancy increased the ratio in WKYs but not in SHRs.

The litter size was slightly but not significantly smaller for SHRs versus WKYs (Table 3). The fetal body weight was significantly lower in SHRs than in WKYs. On the other hand, the placenta was heavier for SHRs versus WKYs. Plasma glucose in the fetus was lower in SHRs compared with WKYs. Plasma insulin in the fetus was slightly but significantly ($P < .05$) higher in SHRs than in WKYs. Moreover, the pooled-pancreas insulin content was greater in SHRs versus WKYs (1.4 ± 0.0 *v* 0.7 ± 0.0 μg/fetus, $P < .05$).

DISCUSSION

We found that pregnancy remarkably reduced blood pressure in both SHRs and WKYs, a finding already reported by others.[15,16] One may argue that our findings are biased because the pregnant rats were not accustomed to the measurement of blood pressure. Since all virgin rats in this experiment were accustomed to the manipulation before mating, we believe that accustomation would at most marginally influence our findings.

Until the present time, there were no available data to explain the relationship between insulin resistance and blood pressure regulation during late gestation in both strains of rats. We have clearly demonstrated that a decrease in blood pressure with pregnancy cannot be explained by a change in peripheral insulin resistance.

Next, the influence of pregnancy on insulin secretory activity was examined in SHRs and WKYs. Pregnancy markedly augments insulin release induced by glucose and arginine in vitro and pancreatic insulin stores in Wistar rats.[17] We also found an exaggerated insulin secretory activity in response to glucose in male SHRs in vitro.[18] The glucose-induced insulin response was low and was not enhanced in SHRs (Fig 1). This finding may be associated with the fact that the insulin content of the pancreas was not increased in pregnant SHRs (Table 2). The replicative capacity of pancreatic β cells after partial pancreatectomy was shown to be impaired in male SHRs compared with male WKYs.[19] From the present study, it is possible to speculate that the replicative capacity of the pancreatic islet in pregnancy may be hampered in female SHRs. This hypothesis can be verified by the same type of experiment as demonstrated in male SHRs.[19]

Pregnancy reduced blood pressure in SHRs and WKYs. A decrease in blood pressure near term is not associated with an alteration of insulin resistance in either strain of rats. One possible explanation may be an increase in nitric oxide in pregnancy,[20] although further study is clearly needed.

The litter size and fetal body weight were lower in SHRs versus WKYs. Since the pregnancy weight between SHRs and WKYs differed by approximately 20% (Table 1), this may have influenced the fetal weight. On the other hand, placental weight was greater in the former versus the latter. These results are partially compatible with a recent epidemiological study[11] showing that retarded growth in fetal life was strongly correlated with high blood pressure in adult life.

We recently found that retarded fetal growth due to a low-protein diet during gestation and lactation in SHR dams did not affect hypertension in adulthood.[21] Thus, it may not be

Table 3. Litter Size, Body Weight, Placent Weight, Plasma Glucose, and Plasma Insulin in Fetuses From WKY and SHR Dams

Group	Litter Size	Body Weight (g)	Placenta Weight (g)	Plasma Glucose (mg/dL)	Plasma Insulin (ng/mL)
WKY (n = 12)	10.2 ± 0.5	3.4 ± 0.0	0.36 ± 0.0	75 ± 4	4.1 ± 0.3
SHR (n = 14)	9.4 ± 0.8	3.3 ± 0.0†	0.40 ± 0.0†	36 ± 2†	5.0 ± 0.3*

NOTE. Fetal plasma glucose and insulin were determined with pooled plasma from fetuses in a single litter. Numbers in parentheses are the number of litters. Values are the mean ± SE.
*$P < .05$, †$P < .0001$ *v* WKY.

appropriate to test the hypothesis described by Barker[11] using SHRs. Churchill et al[22] reported a continuous inverse association between fetal growth and maternal blood pressure in normal pregnancy. However, more extended studies are needed to confirm their observations.

Pinilla et al[12] demonstrated various alterations of reproductive function in SHRs such as a reduction in the pregnancy success rate and a decrease in the number of fetuses born and their survival after birth. These findings are somewhat similar to the data for fetuses obtained from diabetic dams.[23]

On the other hand, plasma insulin levels and pancreatic insulin stores in fetuses were greater in SHRs compared with WKYs. Isolated fetal islets from SHR dams secreted significantly more insulin in response to nutrient secretagogues versus islets from WKYs (K. Tanigawa and M. Kawaguchi, unpublished observation, 1995). It is therefore possible to speculate that fetal hyperinsulinemia in SHRs may be linked to the development of hypertension in adulthood. Since hyperinsulinemia during fetal life is associated with accelerated fetal growth,[24] the fact that fetal weight was lower in SHRs versus WKYs remains unexplained. Placental dysfunction due to microangiopathy adversely affects fetal development in diabetic pregnancy.[25-27] Glucose flux via the placenta and/or uteroplacental blood flow may be decreased in SHRs. Therefore, it is necessary to study the function and structure of the placenta in both strains of rats.

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