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Is the rat a proper model for studying human diabetogenic tendencies in pregnancy?

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Human pregnancy produces diabetogenic realignments, as shown by diminished hypoglycemic effectiveness of insulin and augmented peripheral insulin breakdown. The rat has been used as a model for studying these relations, since it also has a subnormal hypoglycemic response to i.v. insulin. Responsiveness to insulin was determined in fat pad pieces from fed and 48-h fasted, 19-day pregnant rats and virgin controls, incubated with U-[^{14}C]-glycerol. Effects of insulin, inhibiting the release of glycerol and synthesis of [^{14}C]-glyceride-glycerol, and promoting [^{14}C]-CO_2 and fatty acid formation from that substrate, were greater in tissues from pregnant than control rats. A pulse of U-[^{14}C]-alanine through the portal vein and collection of blood from the suprahepatic veins gave an index of glucose production, which was suppressed by insulin to a similar extent in pregnant and control rats. After oral glucose (2 g/kg), circulating insulin was higher in pregnant than in virgin rats, but changes in glycemia were similar in both groups. These results demonstrate that in the rat, pregnancy enhances tissue insulin sensitivity and augments B-cell response to insulinotropic stimulus. Therefore, the rat may be not the proper model for studying diabetogenic changes characteristic of human pregnancy, but may be used for the identification of factors protecting against diabetogenic phenomena associated with pregnancy.

Introduction

In the last trimester of human pregnancy, the release of insulin in response to oral or i.v. glucose administration is greatly enhanced[1-4], whereas glucose disposition is either reduced[5-6], or remains within the normal nonpregnant range[7-9]. This indicates an increased resistance to insulin in the mother, which is caused by several factors: (1) presence in plasma of hormones of placental origin, such as progesterone[10], estrogens[11], and lactogens[12,13], which augment islet secretory responsiveness and affect the metabolism of target sites, reducing their sensitivity to insulin action[8,14]; (2) metabolic realignments in late pregnancy, with a shift to lipid metabolism in the mother, as shown by her intense hyperlipidemia[15,16] related to adipose tissue lipolysis[17] and increased hepatic synthesis of very low density lipoproteins[18], as well as the difficulties in lipid transport across the placenta[19,20]. These changes counteract insulin effects and thereby reduce the maternal use of other fuels, such as glucose or amino acids, that more easily cross the placenta; (3) reduced number and affinity of insulin receptors in maternal tissues[21,22].

These factors account for the diabetogenic tendencies in pregnant women, demonstrable by increased insulin requirements in recognized diabetics, the shift toward overt diabetes of previously unidentified diabetics, and the diminished hypoglycemic potency of insulin[23].

Evidence supporting diabetogenic tendencies in the pregnant rat. The rat has been extensively used in studies of many aspects of fuel-insulin interactions during gestation, to simulate disturbances in the pregnant woman. Previously we have shown that the rat model is valid for evaluation of several metabolic parameters. When compared with nonpregnant animals, the late pregnant rat has decreased circulating glucose and increased insulin levels, augmenting the insulin/glucose ratio[24,25], as well as diminished hypoglycemic response to high doses of iv. insulin[26]. It also exhibits intense hyperlipidemia[27,27a], which, among other

factors, is a consequence of enhanced lipolysis[28,29] and reduced lipoprotein lipase activity in adipose tissue[27a,30,31]. Whereas these findings are in accord with the diabetogenic tendencies of human pregnancy, other observations indicate that the generality of this conclusion should be reconsidered.

Results and comment

Findings indicating that pregnancy in the rat is not 'diabetogenic'. Foglia *et al.*[32] reported that pregnancy following 95% panreatectomy does not result in diabetes, compared with nonpregnant control rats, signifying that during pregnancy the rat, unlike man, is protected against diabetes development. To understand this difference, an oral glucose load was given to 19-day pregnant rats and age-matched virgin female controls. Basal blood glucose concentrations were lower and plasma insulin concentrations were higher in pregnant rats than in their controls (Fig. 1) the ratio of insulin/glucose being significantly higher in the former group ($\bar{1}01\pm15$ vs $44\pm\bar{1}6$ μu/mg, respectively, $P<0.05$). After administration of 2 g/kg glucose, the percentage change of both blood glucose and plasma insulin was almost identical in the 2 groups (Fig. 1). The findings indicate that, unlike the reaction in humans, in the

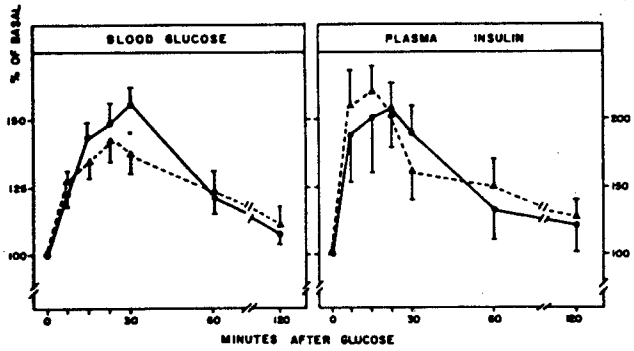
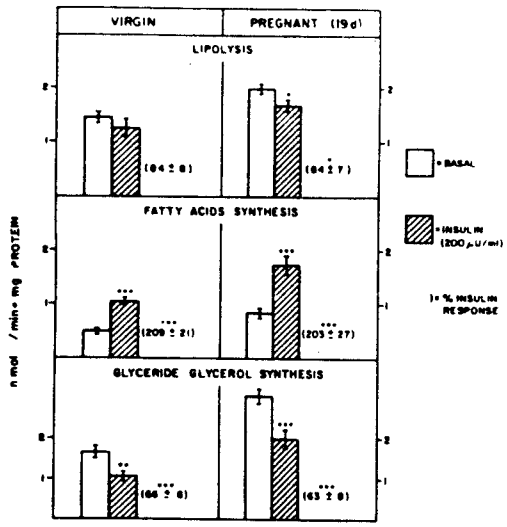


Fig. 1. *Oral glucose tolerance tests (2 g/kg) in virgin (▲—▲) and 19 day pregnant (●—●) rats.* Values are expressed as percentage of those at 0 time (basal) and are means ± s.e. for 6–8 rats per group. *indicates a significant ($P < 0.05$) statistical difference between values in pregnant and virgin animals. Glucose load was given intra gastrically without anesthesia and blood samples were collected from the tip of the tail. Glucose was measured in whole blood[33], after protein precipitation[34]. Insulin was determined in plasma[35] with a radioimmunoassay kit for rat generously provided by Novo Industri A/S (Copenhagen, Denmark). Basal glucose values: 64 ± 6 and 97 ± 2 mg/dl in pregnant and virgin rats respectively ($P < 0.001$). Basal insulin values 77 ± 20 and 45 ± 17 μu/ml respectively (nonsignificant).

rat oral glucose tolerance is unaffected by pregnancy. This conclusion is in agreement with the unchanged glucose disappearance rate after glucose load reported by Leturque *et al.*[36] in the rat during late pregnancy, and it probably results either from: (1) greater pancreatic sensitivity to the insulinotropic stimulus of glucose of the pregnant versus the nonpregnant rat; or (2) unmodified response to insulin in target tissues. The first point has already been substantiated by our results, since at all times after the glucose load, blood glucose concentration remained lower in the pregnant rat than in their virgin controls (absolute integrated values in pregnant animals were 69.3±2.7% of those in virgins, $P<0.001$), while plasma insulin was always higher (absolute integrated values in pregnant rats were 160.3±11.7% of those in virgins, $P<0.001$). As half life of circulating insulin is known to be shorter in the pregnant rat[37], these results may be interpreted as demonstrating that, in spite of lower circulating glucose levels, the pancreas of the pregnant rat secretes a greater amount of insulin. This action coincides with the B-cell hypertrophy and hyperplasia found in the pregnant rat pancreas[38,39] allowing the mother to satisfy her enhanced need of insulin[40].

Tissue insulin response in the pregnant rat. A second explanation for diabetogenic protection during pregnancy in the rat may be that, contrary to humans, tissue responsiveness to insulin is unchanged or even enhanced with gestation. To study this possibility, glycerol metabolism was measured in adipose tissue from 19-day pregnant rats and their virgin con-



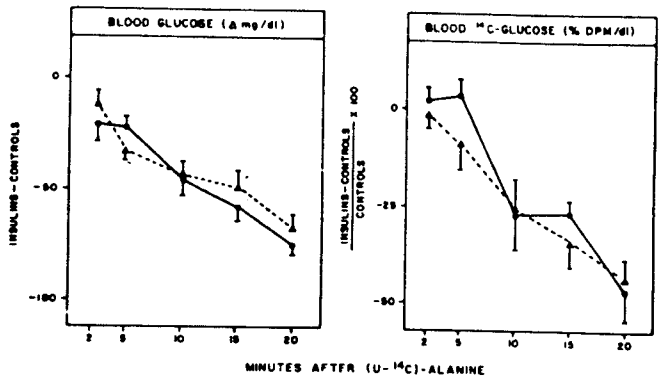


Fig. 2 (Above, left). *Effect of insulin on the rates of glycerol release (lipolysis) and formation of fatty acids and glyceride glycerol, by pieces of lumbar fat pads from 19-day pregnant rats and their virgin controls.* Incubations were carried out in the presence of 10 μu/ml U-[^{14}C]-glycerol (0.5 μCi/ml), 5 mmol/l glucose and purified bovine albumin, as previously described[41]. The rates of glycerol release into the media and its utilization by the tissues were calculated[29,43] and values are expressed as means ± s.e. for 6 rats per group. *indicates significant differences between the groups.

Fig. 3 (Above, right). *Response to intraportal insulin infusion in fed 21-day pregnant rats (●—●) or virgin controls (▲—▲) after a pulse of U-[^{14}C]-L-alanine (200 μmol/200 g, 5 μCi).* The experimental procedure was previously described[51] with 33 mU/min Actrapid-monocomponent insulin (Novo, Denmark) or 0.9% NaCl infused for 20 min just after the tracer. Values are means ± s.e. The response was compared in animals from the same group receiving insulin or 0.9% NaCl by measuring glucose and [^{14}C]-glucose values in blood collected at brief intervals, from a cannula placed in the superior cava vein at the level of the suprahepatic veins.

trols[41]. As shown in Fig. 2, the rate of lipolysis and the formation of both fatty acids and glyceride glycerol from labeled glycerol were greater in tissues from pregnant than from virgin animals when incubated in basal conditions ($P<0.01$ for the difference between both groups for either parameter). The submaximal doses of insulin in the medium (200 μU/ml) significantly decreased the rate of lipolysis only in tissues from pregnant animals (Fig. 2). Insulin also decreased the formation of glyceride glycerol, while it enhanced the formation of fatty acids in tissues from both pregnant and virgin rats. Although the hormonal effect was different in direction and magnitude according to the variable studied, it was always percentagewise the same for tissues from both groups (Fig. 2). These results, together with previous findings showing that insulin promotes the metabolism of glucose in adipose tissue from pregnant and virgin rats to a similar extent, indicate that adipose tissue sensitivity to insulin is unaffected by pregnancy in the rat. This occurs in spite of the enhanced basal lipolysis seen in adipose tissue from pregnant animals which seems to be counteracted by an increase in insulin receptors[43,44], allowing a normal response to the hormone.

Other tissues besides adipose tissue also play an important role in the insulin-glucose interactions. There are conflicting reports concerning insulin sensitivity in skeletal muscle, the glucose metabolism of which may be decreased[45] or unchanged[46] in the pregnant rat. Since the liver is responsible for 80% of the endogenous glucose production during the post-absorption period[47], its response to insulin must also play an important role in insulin-glucose interactions during pregnancy. This question is not yet resolved as some investigators have found a rise in hepatic insulin receptors in pregnant rats[48], while others have reported them to be unchanged[49] or even decreased[50]. To study the hepatic response to insulin we used our recently reported technique[51] in which the anesthetized animal is

infused with insulin through the portal vein just after receiving a pulse of labeled alanine as the gluconeogenic substrate. Since blood circulation through the liver is not interrupted, changes in labeled and nonlabeled glucose in blood, collected from the superior vena cava reflect initially the changes in hepatic glucose production, and at later times are also influenced by differences in peripheral glucose utilization. As shown in Fig. 3, the insulin effect was manifest by a progressive reduction in both nonlabeled and [^{14}C]-labeled glucose, likewise in pregnant and virgin animals. These values were similar in the two groups at all times measured, demonstrating that the response of rat liver glucose production to insulin was unaffected by pregnancy.

Thus, several studies, including the present one, do not show consistent alteration of target tissue sensitivity to insulin in the pregnant rat. This contrasts with the observed lower hypoglycemia after insulin administration in pregnant, compared with virgin rats[26,36]. However, the initial hypoglycemia of the pregnant animal[24], and the more rapid disappearance from circulation of administered insulin in rat pregnancy[37], make it difficult to interpret these changes as a decreased overall sensitivity to insulin.

Conclusions and speculations: Comparison of responses to pregnancy in women and in rats

Most metabolic changes occurring with gestation in women are qualitatively similar to those found in rats, justifying the wide use of this species as an experimental model for metabolic studies in pregnancy. We, as others, have attempted to extend this comparison to the diabetogenic tendencies of pregnancy, but obtained results showing that rats are protected against the development of diabetes in pregnancy. The basis for this difference is not yet known but certain factors clearly participate. In the woman as in the rat, pregnancy enhances the insulinotropic response to food intake (and glucose load), although in the pregnant woman this response is accompanied by increased circulating glucose, whereas this does not occur in the rat, which exhibits an unchanged sensitivity to insulin and/or an augmented proportional increase in the pancreatic response to the same insulinotropic stimulus. Both factors seems to participate in the difference. Using other experimental designs to directly study the insulin response of target tissues, we conclude that sensitivity to insulin is unchanged in the rat during pregnancy. Fewer studies on this matter have been performed in tissues from pregnant women, due to obvious limitations, but insulin sensitivity and insulin receptors have consistently been found to be reduced. The pancreatic response to insulinotropic stimulus in the pregnant rat, appears greater than in the pregnant woman. This is suggested by the fact that the augmented postprandial glycemia in pregnant women always precedes the enhanced amplitude of circulating insulin excursions, whereas in the fed pregnant rat blood glucose levels tend to be reduced while circulating insulin concentrations are consistently augmented.

The basic mechanism for these differences is not yet understood but deserves special attention as this information might allow establishment of suitable protective therapy for pregnant women with diabetogenic tendencies. In conclusion, although the rat is not a proper experimental model for mirroring the human diabetogenic clues in pregnancy, it is the ideal animal to use for identification of the factors that may prevent the development of diabetes in the pregnant woman.

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