ACTA ENDOCRINOLOGICA
65 (1970) 352–360

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CARBOHYDRATE METABOLISM IN PREGNANCY

VII. Insulin tolerance during late pregnancy in the fed and fasted rat

By

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ABSTRACT

The effects of pregnancy and dietary status upon the blood sugar response to exogenous insulin in the rat were evaluated. Unanaesthetized 19-day pregnant, postpartum, and age matched virgin rats were challenged with intravenous insulin (10 U/kg) after unrestricted access to food or fasting for 48 hours. Appropriate control studies were instituted to correct for the effects of 'handling' upon blood sugar. *Fed* as well as *fasted* pregnant rats displayed diminished absolute hypoglycaemic responses to insulin and attenuated rates of blood sugar fall. The relative resistance of pregnant rats to the blood sugar lowering actions of insulin was documented during group comparisons with virgin rats as well as upon re-examination of the same animals postpartum.

The finding of maternal islet cell hyperplasia in all species examined to date (*Rishi et al.* 1969) supports the premise that pregnancy enhances requirements for insulin. While insulin demand may be aggravated by greater insulin de-

This work was supported in part by Research Grant AM-10699 and Training Grant AM-5071 from the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service, Bethesda, Maryland. Please address requests for reprints to Dr. Norbert Freinkel, Northwestern University Medical School, 303 E. Chicago Ave., Chicago, Ill. 60611.

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gradation (*Freinkel & Goodner* 1960), the elevation of plasma immunoreactive insulin even under basal conditions points to the operation of additional mechanisms (*Spellacy & Goetz* 1963; *Kalkhoff et al.* 1964; *Bleicher et al.* 1964). Thus it has been proposed that direct resistance of tissues to insulin action develops with gestation and that the hormones of pregnancy are involved (*Kalkhoff et al.* 1964; *Bleicher et al.* 1964; *Freinkel* 1965).

Recent interest has centered on the placental growth hormone-like principle with luteotrophic properties, placental lactogen (*Josimovich & MacLaren* 1962). In non-pregnant humans, administration of this material can exacerbate diabetes (*Samaan et al.* 1968) and blunt the blood sugar lowering effects of insulin (*Beck & Daughaday* 1967); in non-pregnant rats injection of human placental lactogen elicits ketonaemia (*Friesen* 1965) and enhances insulin release (*Malaisse et al.* 1969; *Martin & Friesen* 1969). However, the situation appears to be more complicated with regard to *endogenous* hormonal activities in the pregnant rat. Whereas plasma levels of placental lactogen increase throughout pregnancy in the primate (*Kaplan & Grumbach* 1965; *Beck et al.* 1965; *Samaan et al.* 1966; *Spellacy et al.* 1966), a luteotrophic-mammotrophic principle is present maximally in rat placenta and plasma only at mid-pregnancy (days 11–13) and cannot be demonstrated in the circulation thereafter (*Ray et al.* 1955; *Matthies* 1967). In further contrast to man, material which cross-reacts immunologically with homologous growth hormone is not increased in plasma in the rat during late gestation (*Schalch & Reichlin* 1966), despite reaction of antibody to human placental lactogen with rat placental extract (*Leake & Burt* 1969).

These apparent differences in placental elaborations between rats and primates prompted an investigation of the hypoglycaemic response to insulin in the pregnant rat. An attenuated response during late gestation in man has long been recognized (*Burt* 1956).

MATERIALS AND METHODS

Age-matched virgin and primiparous pregnant rats were obtained from Charles River Laboratories, Wilmington, Mass., and housed as described elsewhere (*Herrera et al.* 1969*a,b*). Tests of insulin tolerance were performed without anaesthesia by injecting »glucagon-free« insulin* (10 U/kg) via tail vein into virgin and 19-day pregnant animals. The insulin had been diluted with 0.85 % sodium chloride (saline) so that total intravenous injection consisted of 0.5 ml. To assess the effects of injection *per se* and repetitive blood sampling upon blood sugar in unanaesthetized animals, control tests were performed by administering saline alone (0.5 ml) to additional groups. Pregnancy was permitted to continue to term and litters were removed at parturition to eliminate

* Generously provided by Dr. William Kirtley of Lilly and Co., Indianapolis, Indiana.

nursing. Seven days after the initial challenge with insulin or saline (i. e. approximately 4 days postpartum), the same test was repeated. The second challenge was designed to provide paired comparisons within individual animals (i. e. pregnant vs. postpartum) as well as group intercomparisons (i. e. pregnant vs. virgin).

Fed and *fasted* animals were tested. *Fed* rats had been given continuous access to Purina Chow pellets prior to the administration of insulin or saline; *fasted* rats had been deprived of food, but not water, for the preceding 48 hours.

Blood specimens (about 200 μl) were collected drop-wise from the cut tip of the tail immediately before and exactly 4, 8, 12, and 16 min after injection. The drops were introduced with continuous stirring into the recesses of porcelain indicator plates which contained dried heparin. Protein-free filtrates (1:20) were prepared with $Ba(OH)_2$-$ZnSO_4$ (*Nelson* 1944) and analyzed for glucose with glucose oxidase (*Huggett & Nixon* 1957).

RESULTS

Mean ± SEM values for blood glucose prior to the administration of insulin or saline are summarized in Table 1. As reported previously (*Scow et al.* 1964; *Herrera et al.* 1969*b*), blood sugar was diminished significantly even in *fed* 19-day pregnant rats, and near hypoglycaemic levels were observed after 48 hours fast. Changes in blood sugar above or below these baselines, following

Table 1.
Blood Glucose Values Prior to Administration of Insulin or Saline*.

	Fed		*Fasted*		*P:*
	n	Blood glucose (mg/100 ml)	n	Blood glucose (mg/100 ml)	Fed vs Fasted
Pregnant (P)	18	76.9 ± 1.5	17	43.1 ± 0.9	< 0.001
Virgin (V)	19	99.4 ± 1.7	22	64.5 ± 1.1	< 0.001
Postpartum (PP)	17	92.4 ± 1.1	17	68.8 ± 1.4	< 0.001
P:					
P vs V		< 0.001		< 0.001	
P vs PP		< 0.001		< 0.001	

* Mean ± SEM values are summarized above. n denotes the number in each category. Pregnant (P) = rats on day 19 of gestation; Postpartum (PP) = rats rechallenged 7 days later (i. e. approximately 4 days following delivery); Virgin (V) = age-matched nulliparous rats. *Fed* animals were given unrestricted access to food, and *fasted* animals were deprived of food for 48 hours prior to insulin or saline administration.

the intravenous injections of insulin or saline (i. e. △ blood glucose in mg/100 ml) are shown in Fig. 1A and 1 C.

Administration of insulin to *fed* animals caused greater reductions of blood

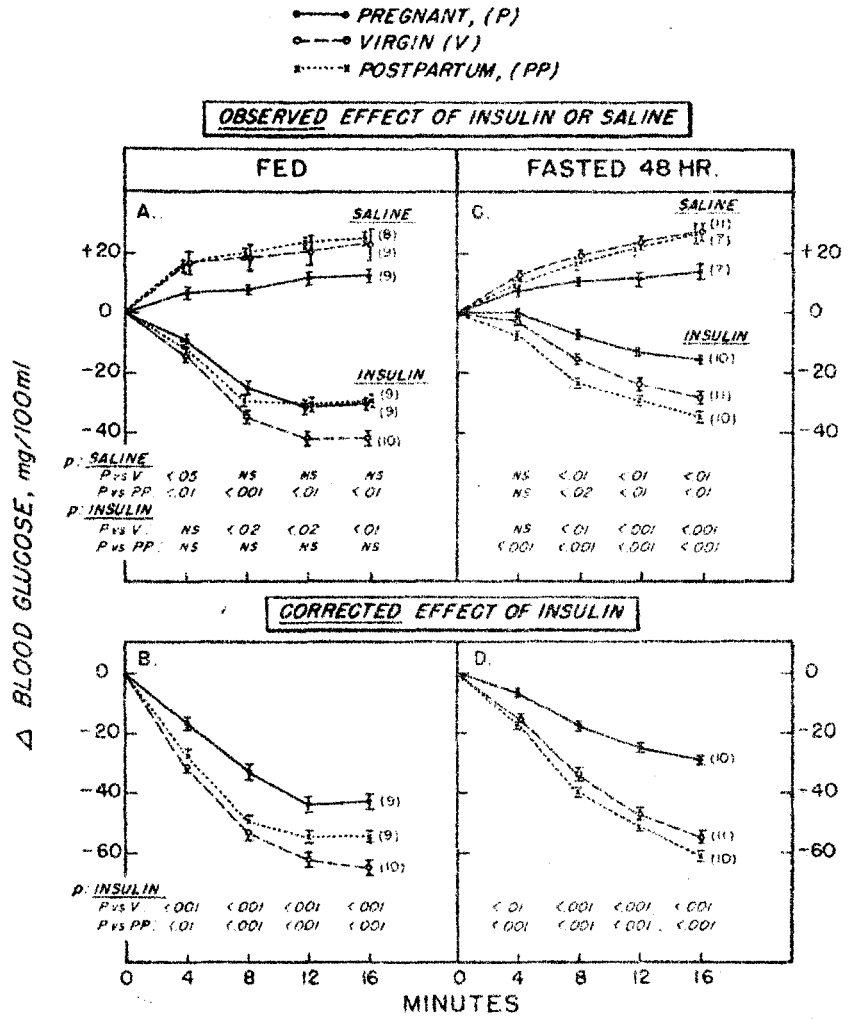

Fig. 1.

Effects of intravenous insulin or saline on blood glucose in the rat: Mean ± SEM changes following the administration of insulin (10 U/kg) or equivalent volumes of saline to pregnant, virgin or postpartum animals are depicted. ( ) denotes the number of animals in each category and *p* indicates the statistical significance of the differences in response between pregnant and virgin (P vs V) and pregnant and postpartum rats (P vs PP). For details see text.

sugar within 8, 12 and 16 min in virgin than in pregnant animals although significant differences between pregnant and postpartum rats could not be demonstrated (Fig. 1A). After 48 hours fast, insulin produced smaller absolute reductions of blood glucose in all groups (Fig. 1 C); hypoglycaemic effects were least pronounced in the pregnant animals (Fig. 1 C).

By contrast, blood sugar levels were increased in all blood samples secured following the administration of saline alone to *fed* (Fig. 1 A) or *fasted* (Fig. 1 C) animals. The increments were least pronounced in the pregnant groups (Fig. 1 A and 1 C)*. To compensate for this hyperglycaemic effect of handling, and to assess the *true* magnitude of insulin action, »corrected« effects of insulin were derived by adding the mean increases in blood sugar obtained after saline to the mean decrements observed after insulin. At all time points in *fed* (Fig. 1 B) as well as *fasted* animals (Fig. 1 D), »corrected« hypoglycaemic effects of insulin were smaller in pregnant than in virgin or postpartum animals ($P < 0.01$–0.001).

To evaluate the acute responsiveness of tissues to insulin before appreciable counterregulation had occurred, regression equations were derived from the »corrected« changes in blood sugar during the first 8 min. Rates of blood sugar fall during this interval (i. e. the slopes of the regression lines) are expressed as mg glucose/ml blood/min and are summarized in Table 2. Rates were significantly less in pregnant than in virgin animals under all conditions. Similar trends were observed when these absolute rates were expressed as a function of the initial blood sugar (Table 1). Thus, during the first 8 min, blood sugar fell an average of 5.28 %, 6.63 % and 6.66 %/min in *fed* pregnant, virgin, and postpartum rats respectively, and 5.15 %, 6.47 % and 7.17 %/min in *fasted* pregnant, virgin, and postpartum rats.

The differences between virgin and postpartum animals (Fig. 1) prompted more detailed evaluation of the insulin tolerance test. Repeated administration of insulin or saline after a 7-day interval to virgin rats elicited virtually identical changes in blood sugar. In view of such reproducibility, differences between postpartum and virgin groups are more likely due to persistent effects of gestation in the postpartum rats than to the effect of repeated testing.

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* The blunted rise in blood sugar after saline injections in the pregnant rat cannot be explained fully from the available data. Although liver concentrations of glycogen (mg/μmole DNA) in pregnant and virgin rats are not significantly different (*Herrera et al.* 1969*b*), total hepatic DNA and glycogen are increased in the pregnant animals almost commensurate to their increases in body weight (*Herrera et al.* 1969*b*). Thus the lesser increase in blood sugar cannot be ascribed wholly to a larger volume for the distribution of glucose released from liver. Utilization of glucose by the placenta and foetus may constitute an additional possibility. Another and perhaps more important factor may be the docility of the pregnant animals and their diminished excitement in response to handling.

Table 2.
Rate of Fall in Blood Sugar during First 8 Min Following Insulin Administration*.

	Fed		*Fasted*		△ (Fed – Fasted)	*P:* (Fed vs Fasted)
	n	k (mg/ml/min)	n	k (mg/ml/min)		
Pregnant (P)	27	–0.0406±0.0031	30	–0.0222±0.0025	–0.0184	< 0.001
Virgin (V)	30	–0.0659±0.0036	33	–0.0417±0.0027	–0.0242	< 0.001
Postpartum (PP)	27	–0.0616±0.0030	30	–0.0493±0.0032	–0.0123	< 0.01
P:						
P vs V		< 0.001		< 0.001		
P vs PP		< 0.001		< 0.001		

* Regression equations were derived by the method of least squares for the »corrected« values in blood sugar during the first 8 min after insulin injection. n denotes the number of coordinates for each line. k = the mean ± SEM values for the slopes of the regression equations and depicts the change in blood sugar as: mg glucose/ml blood/min during the 8 min interval. △ is the difference between mean k values for each group of *fed* and *fasted* rats.

DISCUSSION

Assessment of relative sensitivities to the hypoglycaemic actions of insulin in different groups of intact animals is difficult: full insulin effects may be obscured by differences in counterregulation; responsiveness of individual tissues may not be modified in uniform fashion; and rates of insulin disposition may vary. In this comparison of pregnant and non-pregnant animals, we utilized the acute decrement in blood sugar following the administration of supramaximal doses of insulin as our test situation. The large doses of insulin and the early sampling of blood were designed to minimize the effect of accelerated insulin removal in pregnancy (*Goodner & Freinkel* 1960). In addition, we performed control tests with saline to correct for effects of handling in unanaesthetized animals. Finally, rate constants were derived for net blood sugar changes during the first eight minutes, an interval compatible with analytical precision and yet insufficient for appreciable counterregulation. Within this framework, we have demonstrated that the acute hypoglycaemic response to insulin is blunted in the *fed* as well as in the *fasted* 19-day pregnant rat. The relative resistance of the pregnant animal to the blood sugar lowering effects of insulin was documented during group comparison with age-matched virgin rats as well as upon re-examination of the same animals postpartum.

Strict interpretation is complicated by the fact that basal levels of blood sugar are lower in pregnant rats. However, this association disappears on comparing the *fed* pregnant with the *fasted* virgin or postpartum groups. In this instance, the basal glucose in *fed* pregnant rats is significantly *higher* than in *fasted* non-pregnant animals (Table 1), while the rates of glucose fall in the first 8 min following insulin are statistically indistinguishable (Table 2). In other words, the response of the *fed* pregnant rat to exogenous insulin is like that of the *fasted* non-pregnant rat *despite* a higher initial blood sugar. In addition, when initial blood sugar was taken into account by expressing the 0–8 min rate of glucose fall as a percentage of the initial glucose concentration, the trend toward lower rates for *fed* and *fasted* pregnant rats persisted.

Two aspects of the blunted response of the pregnant rat to the hypoglycaemic action of insulin warrant further evaluation: 1) which tissues are resistant to enhancement of glucose disposition; and 2) which of the hormonal and metabolic changes of pregnancy mediate this tissue resistance.

As yet, the resistant tissues have not been identified. However, *in vitro* studies indicate that adipose tissue may be excluded. We have observed heightened basal and insulin responsive disposition of glucose in association with activated lipolysis during incubation of lumbar fat from *fed* pregnant rats (*Knopp et al.* 1970). *Leake & Burt* (1969) have previously reported that insulin promotes greater uptake of glucose by parametrial adipose tissue from pregnant rats.

The mediation of tissue resistance also remains to be elucidated. Because the resistance can be demonstrated in the fed as well as the fasted state, it is tempting to ascribe it to factors which operate continuously, such as the hormones of pregnancy. As yet, it has not been established whether the small amounts of luteotrophic – mammotrophic peptide (*Astwood & Greep* 1938) demonstrable in the placenta but not the circulation of the rat during late pregnancy (*Ray et al.* 1955; *Matthies* 1967) can exert meaningful metabolic actions. However, the increasing availability of sex steroids might be of major significance. Certain oestrogens and progestins can attenuate the hypoglycaemic effectiveness of insulin in primates (*Beck* 1969; *Beck & Wells* 1969) and alterations in oral glucose tolerance have been elicited in the rat by administration of the synthetic progestin, Norgestrel, alone or in combination with ethinyl oestradiol (*Fenichel et al.* 1969). Finally, it is conceivable that the relative resistance to insulin even in the fed rat may merely represent some persistent metabolic adaptation to the »accelerated starvation« that characterizes the intervals between meals during gestation (*Freinkel* 1965; *Herrera et al.* 1969*a,b*). In any event, it will be important to determine whether the resistance extends to other actions of insulin besides the effects of the hormone upon circulating glucose.

For the moment, therefore, it can only be concluded that the rat, like the primate (*Burt* 1956), exhibits smaller decrements of blood sugar in response to loading doses of *exogenous* insulin during late pregnancy; and that it may therefore require increased delivery of *endogenous* insulin to at least some of the insulin-sensitive sites, to preserve carbohydrate homeostasis. Our recent finding that immunologically-reactive insulin is increased in the circulation of the otherwise metabolically normally fed *19*-day pregnant rat (*Herrera et al.* 1969*b*) is in accordance with this conclusion.

ACKNOWLEDGMENT

The authors are grateful for the excellent technical assistance of Miss Dorothy Turek.

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Received on January 27th, 1970.