

RELATIONSHIPS BETWEEN GLUCOSE, INSULIN AND GLUCAGON DURING FASTING IN LATE  
GESTATION IN THE RAT<sup>1</sup>

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SUMMARY

Plasma glucose, insulin and glucagon were measured in pregnant and age-matched virgin rats in the fed state and after fasting 6, 48 or 120 hours during day 16-21 of gestation. The fed state in pregnancy was characterized by a metabolic setting favoring anabolism. The lower plasma glucose in the fed pregnant rats was associated with higher insulin, slightly lower glucagon and higher insulin/glucose and insulin/glucagon ratios than in virgin rats. During fasting, glucose fell to sustained hypoglycemic levels in the pregnant animals whereas glucose declined but did not achieve hypoglycemia at any point in the virgins. Despite the hypoglycemia, greater levels of plasma insulin persisted in the pregnant throughout the 120 hours of fasting and insulin/glucagon ratios did not differ significantly from the euglycemic virgins. Thus, "accelerated starvation" in pregnancy cannot be ascribed to relative glucagon excess. Rather, the preservation of normal insulin/glucagon ratios despite prevailing hypoglycemia, may provide a mechanism during fasting in pregnancy for restraining maternal protein catabolism in the face of the added fuel demands of the conceptus.

During fasting in late gestation, maternal metabolism is rapidly diverted to the utilization of fatty acids and ketones to sustain oxidative needs, a process we have designated "accelerated starvation" (1). In association with accelerated diversion to lipid fuels, hypoglycemia supervenes but increased urinary nitrogen excretion indicates that augmented protein catabolism occurs also (2). We have suggested that hypoglycemia may pose a threat to maternal homeostasis because the pregnant rat excretes increased amounts of catecholamines in the urine during fasting (3). However, other factors that might

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potentially counteract hypoglycemia have not been examined.

Glucagon has been called a hormone of glucose need and increased plasma concentrations of glucagon and lower insulin/glucagon ratios have been encountered in acute hypoglycemia (4) and in a variety of catabolic states (5-13). Accordingly, it was deemed of interest to examine the relationship between glucose, insulin and glucagon during varying periods of fasting in pregnancy. In normal 30-40 weeks pregnant women we did not observe altered glucagon levels after overnight fast (14) although lower plasma glucose and increased fat mobilization are demonstrable under these conditions. We have been reluctant to fast pregnant women for longer intervals, because of the potentially harmful late consequences of maternal ketosis (15). Therefore, the present studies with pregnant rats were initiated. We have examined the relationships of plasma glucagon to prevailing levels of glucose and insulin during prolonged fasting in gravid and age-matched virgin rats. The limited concurrent observations which appeared while these studies were in progress appear to be in conflict. Plasma glucagon has been reported elevated in four 21 day pregnant rats fasted 96 hours (16) or unchanged in 21 day rats fasted for 48 hours (17).

#### MATERIALS AND METHODS

##### Animals:

Pregnant rats (mated at 47 days of age) and age-matched virgins were received from Charles River Breeding Laboratories, Wilmington, Massachusetts and handled as described previously (2). Pregnant animals with fewer than 8 fetuses were excluded. All experiments with fed or fasted animals were initiated at 8:00 AM on day 16 of gestation.

##### Collection of Specimens and Analyses:

Acute, transient elevations of plasma glucose have been observed in fed rats following pentobarbital anesthesia by Furner et al (18) and Surmaczynska, Metzger and Freinkel (unpublished observations). Concern that anesthesia might effect corresponding distortions of glucoregulatory hormones prompted us to use unanesthetized animals. Accordingly, rats were killed by decapitation with a

guillotine and blood collected into heparinized beakers. An aliquot of blood was added to a solution of Trasylol 5000 units/ml (10/1, v/v) for glucagon analysis. Plasma was separated by immediate centrifugation and frozen at 20° C until analyzed.

Glucose was measured with glucose oxidase (Glucostat, Worthington, Biochemicals) in protein free supernatants of plasma. Plasma immunoreactive insulin (IRI) (19) and immunoreactive glucagon (IRG) (20) were determined in separate double antibody radioimmunoassays using rat insulin and mixed beef-pork glucagon as respective standards.<sup>3</sup> A highly purified preparation of gut glucagon-like-immunoreactive material had less than one-twentieth the potency of pancreatic glucagon when tested with the antiserum used in these assays.

#### RESULTS

Absolute values for plasma glucose, IRI and IRG in pregnant and age-matched virgin rats are summarized in Table I. Derived interrelationships between these parameters - i.e., ratios for IRI/glucose and IRI/IRG are presented in Table II.

#### Glucose:

As reported previously (2, 21) values for plasma glucose in animals fed until sacrifice at 8:00 AM were significantly lower in 16 day pregnant than in virgin rats. To examine a metabolic setting more analogous to the postabsorptive state in man, measurements were also secured in rats 6 hours after withdrawal of food. In nonpregnant animals, no change in plasma glucose was observed. However, in the pregnant, even 6 hours of dietary deprivation resulted in a significant decrement in plasma glucose ( $p < .05$ ). It has been reported that plasma glucose declines to hypoglycemic levels within 24 hours of fasting during late pregnancy in the rat (2, 21). In the present studies, such hypoglycemia persisted when fasts were prolonged to 48 or 120 hours. In nonpregnant animals, corresponding periods of fasting effected a significant fall in plasma glucose

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<sup>3</sup>Purified rat insulin was a gift from D. F. Steiner, M.D., Chicago, Ill. and crystalline beef-pork glucagon was kindly provided by Mary Root, Ph.D., Lilly Research Laboratories, Indianapolis, Indiana.

below fed values. However, the values remained within the normal range throughout 120 hours of food deprivation.

Insulin:

Basal hyperinsulinemia which has been documented during late gestation in normal pregnant women (1) and in fed rats (2) was also found in these studies. Absolute values for IRI (Table I) and IRI/glucose ratios (Table II) were greater in fed pregnant than virgin animals. IRI declined sharply with 6 hours of food deprivation in both pregnant and virgin groups (77% and 69% reductions respectively). Although absolute IRI values were no longer significantly greater than in virgin rats (Table I), IRI/glucose ratios remained higher in the pregnant (Table II). During extensions of fasts from 6 hours to 48 hours, both IRI and glucose declined in virgin animals. Because IRI fell more than glucose, IRI/glucose ratios diminished also (Table II). However, between 48 and 120 hours of fasting, IRI and glucose changed minimally and IRI/glucose ratios plateaued. In pregnant animals, IRI and glucose also decreased between 6 and 48 hours of fasting and then remained constant between 48 and 120 hours (Table I). Thus, their IRI/glucose ratios (Table II), which were already increased after 6 hours of fasting, continued to exceed nongravid values.

Glucagon:

In the fed state, plasma IRG was marginally higher in virgin than in pregnant rats ( $P < .10$ ). In virgins, IRG declined significantly after 6 hours of fasting with additional decrements during fasts extended to 48 and 120 hours. Although plasma IRG fell moderately during fasting, the much greater fall in IRI resulted in a decline in IRI/IRG ratios from fed to 6-hour and 6-hour to 48-hour fasts with no further change between 48 and 120-hours of fasting. In pregnant animals, plasma IRG increased slightly during fasting (Table I). Although absolute levels of IRG were higher in pregnant rats, IRI/IRG ratios did not differ in pregnant and virgin animals after 6, 48 or 120 hour fasts (Table II).

TABLE I  
PLASMA GLUCOSE, IRI AND IRG IN THE RAT  $\pm$

	<u>FED</u>		<u>FASTED</u>	
		6	48 (hours)	120
<u>GLUCOSE</u>				
Pregnant	96.8 $\pm$ 3.0 (6)	85.0 $\pm$ 3.1* (6)	48.2 $\pm$ 2.5*** (7)	49.0 $\pm$ 1.9*** (7)
Virgin	121 $\pm$ 3.4 (7)	118 $\pm$ 2.7 (7)	79.1 $\pm$ 2.2*** (6)	76.8 $\pm$ 5.3*** (6)
P	<.001	<.001	<.001	<.001
<u>IRI</u>				
Pregnant	137 $\pm$ 25 (6)	31.3 $\pm$ 5.2*** (6)	13.9 $\pm$ 2.2*** (7)	15.2 $\pm$ 1.9*** (7)
Virgin	79.5 $\pm$ 19 (7)	24.7 $\pm$ 2.8* (7)	6.6 $\pm$ 0.69*** (6)	5.6 $\pm$ 0.80** (6)
P	<.10	NS	<.01	<.001
<u>IRG</u>				
Pregnant	71.1 $\pm$ 10 (6)	83.7 $\pm$ 11 (6)	81.6 $\pm$ 5.9 (7)	92.4 $\pm$ 4.0 (7)
Virgin	107 $\pm$ 16 (7)	62.6 $\pm$ 6.9* (7)	52.0 $\pm$ 7.6** (6)	44.0 $\pm$ 5.8** (6)
P	<.10	NS	<.01	<.001

$\pm$  Pregnant rats were given unrestricted access to food until 8 AM on day 16 of gestation ("fed") or deprived of food for the subsequent 6, 48, or 120 hours ("fasted"). Age-matched virgin animals were treated similarly. Blood specimens were secured by decapitation as described in the text. ( ) denotes number of animals in each group. p denotes statistically significant differences between pregnant and virgin animals. Asterisks indicate statistical significance of values at each time during the fast compared to those prevailing in the fed state; \*p <.05; \*\*p <.01; and \*\*\*p <.001.

#### DISCUSSION

Elevated levels of plasma glucagon have been reported in catabolic states such as overt diabetes (7,8), glucocorticoid excess (10, 13), or fasting (5, 6). More pronounced, hyperglucagonemia has been found in association with the heightened catabolism of infection (12), trauma (9) or diabetic ketoacidosis (11). Elevations of plasma glucagon have been observed acutely during insulin

TABLE II

IRI/GLUCOSE AND IRI/IRG RATIOS IN THE RAT  $\pm$ 

	<u>FED</u>		<u>FASTED</u>		120
		6	48	(hours)	
<u>IRI/GLUCOSE</u>					
	(μU/mg)				
Pregnant	142 $\pm$ 25	36.7 $\pm$ 6.1***	29.6 $\pm$ 5.2***	31.3 $\pm$ 4.2***	
Virgin	64.4 $\pm$ 14	21.0 $\pm$ 2.5**	8.4 $\pm$ 0.91***	7.4 $\pm$ 0.89***	
p	<.02	<.05	<.001	<.001	
<u>IRI/IRG</u>					
	(molar ratio)				
Pregnant	46.1 $\pm$ 10.8	9.6 $\pm$ 2.0**	4.0 $\pm$ 0.77**	3.5 $\pm$ 0.32**	
Virgin	22.8 $\pm$ 9.4	8.9 $\pm$ 0.84	2.9 $\pm$ 0.35**	3.1 $\pm$ 0.57**	
p	<.05	NS	NS	NS	

$\pm$  Ratios were derived from the values summarized in Table I. Statistical analyses were carried out after logarithmic transformation of the ratios. p denotes statistically significant differences in values in pregnant vs. virgin. Asterisks indicate statistical significance of values at each time during the fast compared to those prevailing in the fed state; \*p <.05, \*\*p <.01, \*\*\*-p <.001.

induced hypoglycemia (4). Finally, glucagon is catabolic when administered chronically to pregnant (22, 23) or nonpregnant (24) animals.

Thus, it might have been predicted that hyperglucagonemia and greatly reduced IRI/IRG ratios would be observed in association with accelerated catabolism and hypoglycemia during fasting in pregnancy. Instead, plasma IRG was increased only minimally compared to levels observed in the fed state and this was balanced by higher IRI levels. IRI/IRG ratios were virtually identical in the hypoglycemic pregnant and euglycemic virgin rats fasted as long as 120 hours. Therefore, some factor(s) other than glucagon, perhaps originating in

the conceptus, must mediate the "accelerated starvation" in the pregnancy.

The failure of plasma IRG to increase and IRI to fall in association with fasting hypoglycemia indicates that pancreatic islets do not respond to the hypoglycemia in a way that would be expected to maximize counterregulatory glucose production. Indeed, it is conceivable that the pancreatic hormones may be reacting in an interdependent fashion. Thus, the "extra" IRI that is persistently elaborated even during fasting in late pregnancy could be obtunding the alpha cell responsivity to hypoglycemia. In any event, the net result of this insulin may be to restrict the magnitude of maternal catabolism thereby preserving protein stores with maternal hypoglycemia as the price that must be paid.

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