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Metabolic Realignments in Late Pregnancy: A Clue to Diabetogenesis*

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The Nature of the Problem

The general features of carbohydrate metabolism during pregnancy may be summarized as follows (*1*): in the pregnant female with adequate pancreatic reserve, tolerance to intravenous glucose is normal whereas the hypoglycemic response to exogenous insulin is subnormal. The hypoglycemic effectiveness of endogenous insulin also may be diminished as judged by the subnormal lowering of blood sugar after intravenous tolbutamide. The dichotomy would suggest that an enhanced elaboration of endogenous insulin is required to maintain normal glucose tolerance during pregnancy. The histological finding of islet cell hyperplasia during normal gestation, and the clinical experience in subjects with marginal or absent pancreatic islet cell reserve are in accord with this premise.

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The changes in maternal carbohydrate metabolism seem to parallel the growth and perfusion of the conceptus (*1*). Thus, diabetogenic influences are insignificant in the first trimester and maximal in the seventh month. Moreover, postpartum, within hours after expulsion of the conceptus, the hypoglycemic effectiveness of insulin and tolbutamide may return to normal, normoglycemia may supervene in prediabetic subjects, and therapeutic insulin requirements may decline acutely in the known diabetic.

Clearly, more catholic clues concerning diabetogenesis may be derived from an analysis of how the conceptus affects maternal carbohydrate homeostasis (*2*).

Metabolic Properties of the Conceptus

The Role of the Conceptus in Insulin Degradation

Our earliest efforts were addressed to the possibility that the conceptus may increase maternal insulin requirements by acting as an added site for insulin degradation (*1, 2*). Our findings may be summarized as follows: Pregnancy does not alter the insulin-degrading capacity of extrauterine structures. However, both portions of the conceptus, i.e., the fetus and placenta, contain proteolytic enzymes for cleaving insulin into nonhypoglycemic products. These systems are present throughout gestation and their total potential activity increases in parallel with the size of the conceptus. Not all have access to maternal insulin since the placenta in the rat, and probably in the human, is relatively impermeable to insulin. However, even the placental contribution may be meaningful since the fractional rate of removal of maternal insulin is significantly accelerated over wide ranges of plasma insulin, near term, and again restored to normal in the immediate postpartum period (*1, 2*).

Other Contributions from the Conceptus

If the requirements for "extra" insulin during pregnancy were due solely to increased degradation, one would anticipate that "steady-state" levels of plasma insulin should be normal after a new equilibrium between insulin secretion and insulin breakdown had been established. On the other hand, additional factors would have to be invoked if plasma insulin were elevated. This proved to be the case: In confirmation of Spellacy and Goetz (*3*), several laboratories demonstrated that plasma immunoreactive insulin after overnight fast is significantly increased in late human pregnancy (*4, 5*). Moreover, by examining normal women in the last trimester of pregnancy, and a week or

more following delivery, we observed that the concomitant plasma levels of glucose are significantly lower, and free fatty acid (FFA) significantly higher antepartum than postpartum (*4*). When we challenged these women with 25 gm glucose intravenously, a substantially greater acute rise in plasma insulin occurred antepartum than postpartum despite smaller increases in blood sugar (*4*). The "extra" insulin did not increase the fractional rate of glucose disposition (i.e., K, percent per minute) (*4*): The unchanged K, even in the presence of the conceptus as an added site for glucose removal, suggested that the maternal utilization of glucose at extrauterine sites actually may have been reduced (*2, 4*).

The foregoing evidence for some antagonism to insulin action prompts consideration of two other aspects of the conceptus:

The Conceptus As an Endocrine Structure

Both fetus and placenta elaborate hormones. However, the small size of fetal endocrine structures and the limitations in transplacental passage diminish the likelihood that fetal hormones exert an appreciable action within the mother. On the other hand, an active biosynthesis of steroid and peptide hormones within the placenta has long been recognized, and these principles have direct access to the maternal circulation. In the least, estrogens, progesterone, chorionic gonadotropin, and placental lactogen warrant consideration (although it is still not clear whether placental lactogen is present in *late* pregnancy in certain species, such as the rat). The actions of these hormones upon extrauterine metabolism are presently being clarified. However, it is significant that elevations of plasma insulin have been found in normal subjects after the exhibition of oral contraceptive agents (*6*), and that mobilization of fat and impairment of carbohydrate tolerance have been produced in humans by administering placental lactogen under appropriate circumstances (7–9). Apparently, some, if not all, of the placental hormones may exhibit contrainsulin actions. More importantly, the limited available studies indicate that the placental hormones are not subject to metabolic feedback regulation. Instead, they continue to be elaborated into the maternal circulation independent of maternal eating, fasting, and so forth.

The Conceptus and Fuel Conservation

During starvation in the nongravid state, peripheral caloric needs are increasingly met by the metabolism of fat. After full adaptation, endogenous glucose is reserved for the oxidative demands of the central nervous system which cannot be met by alternative fuels. Such parsimony is not possible in pregnancy. As shown in Table I, fetal weights are the same in 19-day pregnant rats whether the mothers are fed or fasted for 48 hours prior to

TABLE I

EFFECT OF 48-HOUR FAST (DAY 17 TO DAY 19 OF GESTATION) ON FETAL BODY WEIGHT IN THE PREGNANT RAT[a]

	Initial weight (gm)	Final weight (gm)		p
		Fed (6)	Fasted (6)	
Mother	290.2 ± 4.1 (12)	322.6 ± 3.4	250.3 ± 1.9	<0.001
Fetus	(0.55)	1.81 ± 0.20	1.76 ± 0.11	N.S.

[a] Fetal weights represent values for the average fetus in each litter. Fetuses per litter ranged from 9–12.

sacrifice (*10*). Apparently, the abstraction of maternal fuels continues unremittingly.

What fuels are employed? Esterified fats cross the placenta slowly, and in species such as the rat and human, the transplacental passage of FFA may be limited (*11*). Although ketones readily traverse the placenta (*11*), they cannot provide more than 2-carbon fragments. Other building blocks must be provided for anabolic esterification of fatty acids, glycogenensis, and protein synthesis within the fetus. Such building blocks as glucose cross the placenta freely, and amino acids are actually concentrated in the fetus against a transplacental gradient (*11*). Maternal amino acids are further depleted by heightened urinary losses.

Thus, the mother can conserve neither her glucose nor the amino acids with which to effect gluconeogenic repair. Instead, she must divert extrauterine structures to the utilization of products of fat metabolism at a more rapid rate so as to preserve sufficient glucose for her own central nervous system.

Obviously, the latter contribution of the conceptus will function intermittently in accord with feeding patterns. Loss of maternal fuels poses no problems as long as the mother eats; the challenges to fuel conservation become meaningful only when food is withheld.

Metabolic Changes in the Pregnant Rat

RELATIONSHIPS BETWEEN FUELS AND INSULIN

Some new insights may be derived from recent, and as yet unpublished, studies performed in our laboratory. As a model in which more heroic manipulations can be performed than in the human, we have employed the pregnant

rat. How do the relationships between plasma fuels and insulin in the rat simulate the human? Table II summarizes measurements secured on day 19 of their 22-day gestation period. Animals had been fed or fasted for 48 hours, and blood was drawn from the aorta under Nembutal anesthesia (40 mg/kg). Presumably, the fed values should reflect the *constant* effects of the conceptus whereas the values in fasted animals should reveal the *added* effects of the continued parasitization of *endogenous* maternal fuels. In confirmation of Scow (*12*), we observed lower plasma glucose and higher plasma FFA in pregnant than in age-matched virgin females under both conditions. Moreover, the fast for roughly 10% of the total gestation periods results in near hypoglycemia in the mother (*10*).

In the fed 19-day pregnant rat, plasma insulin is higher than in the nonpregnant animals (Table II). After a 48-hour fast, the levels in the pregnant rat no longer differ significantly although the significant increases in the ratio of plasma insulin/glucose persist. Plasma ketones disclose some important differences between fed and fasted states. Despite higher levels of FFA, the fed pregnant rats maintain lower plasma ketones than control animals (Table II). This would suggest that something is driving lipolysis which is not offset

TABLE II

EFFECT OF PREGNANCY ON CIRCULATING FUELS AND INSULIN IN FED AND 48-HOUR FASTED RATS

	Day of gestation		
	0	19	*p*
Fed rats			
Plasma			
Glucose (mg/100 ml)	109 (10)	81 (10)	<0.001
FFA (μEq/L)	310 (7)	516 (7)	<0.02
Ketones (μEq/L)	285 (7)	145 (7)	<0.01
Insulin (μU/ml)	20 (10)	34 (10)	<0.02
Insulin/Glucose	18 (10)	42 (10)	<0.001
48-Hour fasted rats			
Plasma			
Glucose	97 (11)	51 (8)	<0.001
FFA	443 (7)	739 (7)	<0.001
Ketones	1781 (7)	6473 (7)	<0.001
Insulin	10 (11)	13 (8)	N.S.
Insulin/Glucose	10 (11)	24 (8)	<0.001

* Mean values are summarized above. Numbers in parentheses indicates number of animals in each group. *p* Denotes statistical significance of the differences between groups.

by the "extra" insulin, although the insulin acts within the liver to divert the FFA to products other than ketones. On the other hand, in the fasted animals, hepatic ketogenesis appears less restrained than in nongravid rats.

Does the "extra" insulin represent an attempt to compensate for insulin resistance? An indirect answer was sought by the administration of crystalline insulin (10 U/kg), or equivalent volumes of saline, to fed and 48-hour fasted rats on day 19 of gestation, and again 4 days postpartum (i.e., 26 days after mating). Similar studies were also performed in age-matched virgin females. As judged by area analysis of the integrated fall in blood sugar the responses to insulin in the fed state are smaller antepartum than in postpartum or virgin animals. With fasting, nongravid animals also display diminished responsiveness to insulin. However, the resistance is far more pronounced in the pregnant group. In other words, the constant baseline contributions of the conceptus appear to antagonize the hypoglycemic actions of insulin in the rat, as in the human (*13*). The contributions are exaggerated when dietary deprivation is added.

Lipolysis and Esterification in Isolated Fat

What is happening in adipose tissue? "Steady-state" values for FFA and glycerol in lumbar fat from fed and 48-hour fasted 19-day pregnant rats are shown in Table III and contrasted to similar measurements in virgin females. In both situations, adipose tissue from pregnant animals contains more FFA. However, the disparity between gravid and nongravid animals is more pronounced after fasting. For fuller documentation, segments of lumbar fat were

TABLE III

Effect of Pregnancy on Rat Adipose Tissue Metabolism: "Steady-State" Levels of FFA and Glycerol *in vivo*

	Glycerol (μmoles/mg tissue protein)	FFA (μmoles/mg tissue protein)
Fed rats		
Pregnant*	0.012 ± 0.001 (6)	0.258 ± 0.038 (6)
Virgin	0.011 ± 0.002 (6)	0.147 ± 0.023 (8)
p	N.S.	<0.05
48-Hour fasted rats		
Pregnant*	0.036 ± 0.003 (8)	0.560 ± 0.036 (6)
Virgin	0.020 ± 0.002 (12)	0.222 ± 0.014 (8)
p	<0.01	<0.001

* 19 days pregnant

incubated in KRB containing 28 mg/ml fat-free albumin (KRB-alb) alone, or in association with glucose (3.75 m*M*), or glucose plus insulin (50 μU/ml). Net esterification and lipolysis were calculated as per Vaughan and Steinberg (*14*) (Fig. 1). Both aspects of fatty acid turnover are markedly increased in late pregnancy. Adipose tissue from fed pregnant animals behaves as if it were being driven by some activator(?s) of lipolysis, and this significant distinction persists even when lipolysis is restrained and esterification is promoted by adding insulin and glucose to the suspending media (Fig. 1). The pattern is exaggerated by prior starvation.

Gluconeogenesis

To assess gluconeogenic efficiency under these conditions, 1 mmole of pyruvate-3-^{14}C was administered intravenously to 19-day pregnant and age-matched virgin rats which had been fed or fasted for 24 or 48 hours (*10*). Comparable studies were also performed with 21-day pregnant rats which had been fasted 48 hours (*10*). The percentage of administered radioactivity subsequently recovered as circulating glucose-^{14}C is shown in Fig. 2. In fed animals, pregnancy did not alter the net transformation of pyruvate to glucose

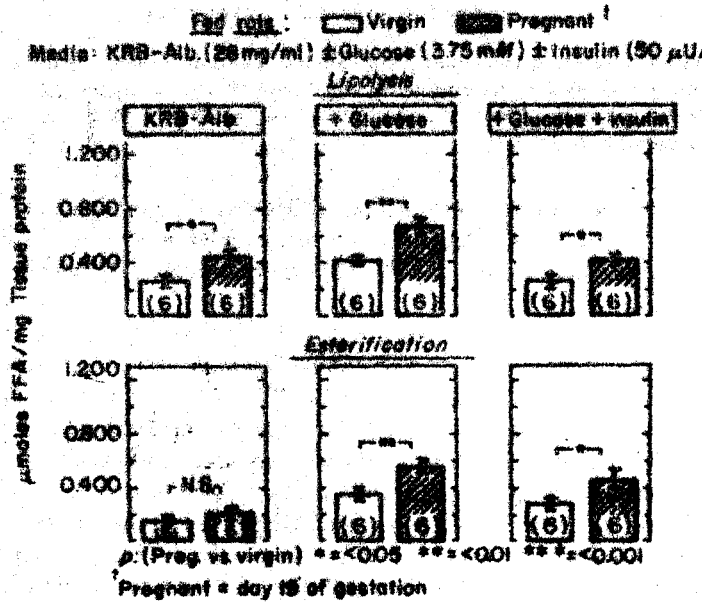

FIG. 1. Effects of late pregnancy on lipolysis and esterification in isolated segments of lumbar adipose tissue from fed rats. Incubations were performed for 150 minutes as described in text.

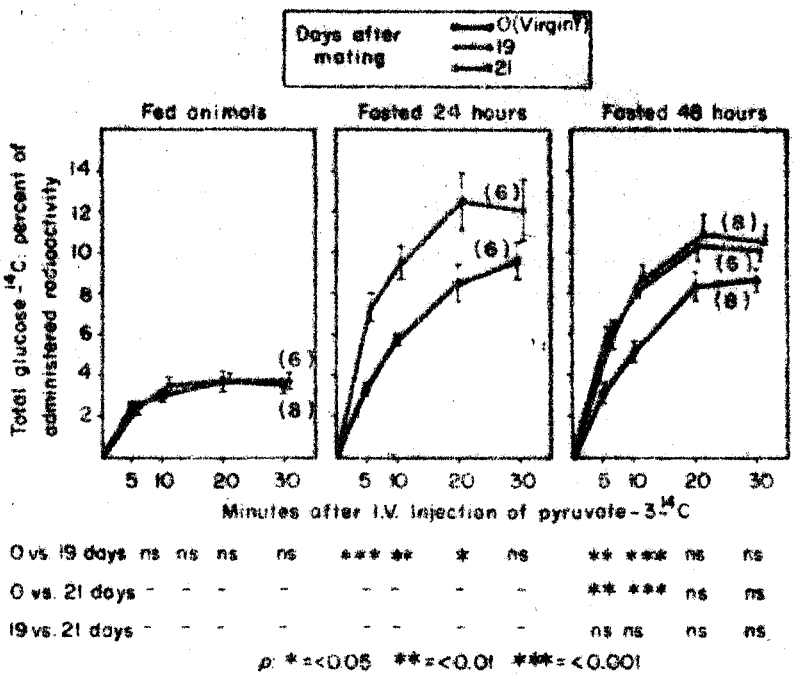

FIG. 2. Effectsof late pregnancy on the formation of glucose-^{14}C from pyruvate-3-^{14}C; significant differences between fed and fasted virgin, and 19 and 21 day pregnant rats are indicated in the lower portion of the illustration. The number in parentheses denotes number of animals in each group.

indicating that the "extra" insulin may offset any intrinsic gluconeogenic potentialities of placental hormones. However, after 24 or 48 hours of starvation, pregnant animals formed significantly more glucose-^{14}C within 5 and 10 minutes following injection (*10*).

The "loading" doses of pyruvate employed for these experiments should have displaced endogenous fuels and eliminated variables as differences in precursor pool size, etc. (*15*). Nonetheless, additional studies were performe to examine pure endogenous events (*10*). Urine specimens were collected from the same animals during access to food for 1 day and subsequent starvation for 2 days on days 18–21 of gestation and days 9–12 postpartum. Similar collections were also secured in age-matched virgin rats. In the fed state, none of these groups displayed significant differences in their urinary excretion of urea, creatinine, or uric acid. Significant differences became manifest during starvation (Table IV).

In the pregnant animals, total urinary N was significantly greater during the first as well as the second day of starvation. However, on the first day, urea N

TABLE V

THE EFFECT OF PREGNANCY ON THE URINARY EXCRETION OF NITROGEN (mg N/24 HOURS) DURING FASTING IN THE RAT[a]

			p		p
		Virgin		Pregnant	Post Partum
	n:	5		8	8
Fasted 24 hours					
Total N		220.0	<0.01	285.5	<0.01	209.8
Urea N		173.8	<0.05	221.2	<0.02	165.8
Uric acid N		0.86	<0.02	1.17	<0.01	0.84
Creatinine N		3.42	N.S.	3.14	N.S.	2.92
Ammonia N		12.9	<0.01	17.8	<0.001	9.1
Fasted 48 hours					
Total N		179.6	<0.01	233.4	<0.01	171.5
Urea N		138.7	N.S.	142.6	N.S.	124.9
Uric acid N		0.88	N.S.	0.90	N.S.	0.87
Creatinine N		3.43	N.S.	3.18	N.S.	3.11
Ammonia N		17.9	<0.001	44.2	<0.001	15.3

[a] Mean values for urinary excretions during first and second day of fasting are summarized above. Statistical significance of the differences between groups were derived on the basis of Mean ± SEM values. Pregnant animals were fasted from day 19–21 of gestation and again on days 10–12 postpartum. Comparable collections were secured during 48-hour fasts in age-matched virgin eats.

accounted for most of the differences, attesting to heightened gluconeogenic transformations within the liver. During the second day, the significant differences for individual N-containing components were confined to ammonia (Table IV) (*10*). Considerable recent work has suggested that urinary ammonia may reflect renal gluconeogenesis to provide a fixed base for the excretion of organic acids (*16*). That premise was corroborated by the highly significant correlation which we observed between urinary NH_4^+ and urinary ketones in the fasted pregnant animals. Thus, despite the increased levels of circulating FFA, the pregnant mother, when fed, does not increase her gluconeogenesis. When fasted, she meets the challenge to her carbohydrate reserves by increasing the net efficiency of her gluconeogenic performance and by activating hepatic and renal mechanisms more rapidly.

Conclusions and Speculations

How can all this be integrated into a cohesive format? One may begin by assuming that the placenta elaborates hormones with contrainsulin

properties. Their secretion parallels the growth of the conceptus and is not subject to metabolic feedback regulation.

At present, one cannot assign relative importance to individual hormones, nor can one say whether any, or all, *directly* act upon islets, muscles, or liver. However, in concert, they effect an enhanced turnover of fat in adipose tissue, and they increase the amount of insulin that is required for glucose disposition in the intact animal. In the fed state, the mother elaborates sufficient insulin to maintain glucose homeostasis and to restrain gluconeogenesis and ketogenesis. However, when food is withheld, plasma insulin falls and opposition to the persistent contrainsulin factors is removed. The fall of insulin may even be accelerated by its continued degradation within the placenta. Thus, ketogenesis, gluconeogenesis, and all the adaptations to starvation are "triggered" more rapidly. Extrauterine mechanisms may be supplemental. For example, we have observed increased elaboration of catecholamines as blood sugar in the fasted pregnant rat approaches the hypoglycemic range (*17*).

What physiological ends are subserved in this fashion? One could suggest that the potentiality for "accelerated starvation" is protective for mother as well as fetus. With an intermittently eating mother and a continuously feeding fetus, a metabolic setting for rapid transfer to fat would be highly desirable. It would afford maximal conservation of maternal glucose and gluconeogenic precursors when exogenous nutrients are withheld and assure their availability for the maternal brain and fetal tissues. The increasing placental elaboration of contrainsulin factors in parallel with the growth of the fetus provides just the right temporal juxtaposition to make it all work.

Thus, the "extra" insulin which is required for anabolism whenever the mother eats represents the overhead that she must pay for survival in the fasted state. When her pancreas cannot meet the payments, diabetes supervenes.

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