Carbohydrate Metabolism in Pregnancy

XI. Response of Plasma Glucagon to Overnight Fast and Oral Glucose during Normal Pregnancy and in Gestational Diabetes

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SUMMARY

Plasma glucagon was examined after overnight fast and in response to 100 gm. oral glucose in sixteen subjects with normal carbohydrate metabolism and in ten gestational diabetics during week 30 to 40 of pregnancy and again five to eight weeks postpartum. In comparison to nulliparous, nongravid subjects, plasma glucagon after overnight fast was not significantly changed antepartum. However, on a pair-matched basis, small but significant and as yet unexplained reductions in plasma glucagon were evident during the postpartum period. Thus, hyperglucagonemia cannot be implicated in the accelerated starvation that is already manifest after overnight fast in late pregnancy, and altered basal glucagon does not constitute one of the diabetogenic factors of gestation. Following glucose administration, plasma glucagon in normal subjects fell to a greater degree antepartum than postpartum. The paired observations suggest that this heightened suppressibility of circulating glucagon may be linked to the more prolonged hyperglycemia and hyperinsulinemia that occur during normal oral glucose tolerance in late pregnancy. In the gestational diabetics, oral glucose also elicited suppression of plasma glucagon antepartum, whereas suppressibility was not seen postpartum. Therefore, overt diabetogenesis in vulnerable subjects during pregnancy cannot be ascribed to lack of alpha cell suppressibility by glucose. Conversely, the exaggerated hyperinsulinemia and hyperglycemia in response to oral glucose during late pregnancy in gestational diabetics may obscure an intrinsically diminished sensitivity of their alpha cells to glucose. DIABETES *23*:771-76, September, 1974.

The response to glucose in late pregnancy is attended by a heightened outpouring of immunoreactive insulin (IRI) which has been known for a decade.[1-3] It has also been recognized that plasma IRI[1,2] as well as insulin-like activity[4] are increased after overnight fast in late pregnancy even though blood sugar levels are lower than in nongravid subjects.[2,5,6] These altered relationships between glucose and insulin during gestation, and the well documented attendant hyperplasia of pancreatic islets,[7,8] prompted us to evaluate the effects of pregnancy on plasma glucagon after overnight fast and in response to glucose. Our interest was heightened by the evidence that pregnancy accelerates many of the metabolic responses to fasting (such as mobilization of fat, activation of gluconeogenesis and ketogenesis),[9] and that increases of plasma glucagon may abet these catabolic processes in other situations, just as reductions of plasma glucagon after carbohydrate meals may enhance anabolism in the fed state.[10-13]

The present report presents the first studies in human beings of glucagon during late pregnancy. Each subject served as her own control by being evaluated during week 30 to 40 of pregnancy (antepartum) and again five to eight weeks following delivery (postpartum). Our efforts included subjects with normal carbohydrate metabolism throughout pregnancy as well as those with gestational diabetes. Preliminary accounts of our findings have been reported previously.[14,15]

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Accepted for publication May 27, 1974.

METHODS AND MATERIALS

Subjects: Pregnant subjects were selected from the Obstetrics Clinic of Northwestern University Medical School and evaluated medically by one of the authors (RRD, BEM, NF or MN). Gestational diabetics were drawn from the population in whom elevations of blood sugar greater than 150 mg. per 100 ml. one hour following screening tests with 50 gm. of glucose by mouth created an index of suspicion. Definitive diagnoses of gestational diabetes were established according to the criteria of O'Sullivan and Mahan[16] following conventional three-hour tests of oral glucose tolerance.

Test Conditions: For the antepartum and postpartum evaluations reported here, tests were performed on an outpatient basis at the Clinical Research Center of Northwestern Memorial Hospital. The tests were initiated between 8 and 9 a.m. after a fourteen-hour fast and a diet containing no less than 250 gm. carbohydrate for each of the preceding three days. The subjects remained recumbent for thirty minutes before and during the three-hour test. Multiple blood samples were drawn without stasis through an indwelling venous needle kept patent between samples with a slow saline drip. Specimens were obtained ten and two minutes before, and 15, 30, 60, 120 and 180 minutes after the ingestion of 100 gm. of glucose dissolved in 300 ml. of water. Aliquots of blood were added immediately to tubes containing Trasylol and EDTA for glucagon determinations. Additional aliquots were introduced into heparin-containing tubes to measure plasma IRI, or permitted to clot to estimate serum glucose. All specimens were chilled on ice, centrifuged at 4° C., and plasma or sera were frozen at −20° C. until analyzed.

None of the subjects were nursing at the time of the postpartum tests.

Analytical Methods: Serum glucose and plasma IRI were measured in Chicago as described previously.[17] Plasma immunoreactive glucagon was determined in Dallas using antibody 30K, which shows minimal crossreactivity with glucagon-like immunoreactivity of gastrointestinal origin, and employing crystalline beef-pork glucagon for standards.[18] Within a single run, the precision of the glucagon immunoassay was such that a difference of more than 10 pg./ml. could be accepted with greater than 95 per cent confidence as being of nontechnical origin. To minimize the contributions of interassay variation, all ante- and postpartum samples from a given subject were assayed for glucagon or insulin in the same assay. Analyses for glucagon were performed on a blind basis, i.e. data concerning glucose or insulin values were not communicated prior to assay.

Results were compared on the basis of paired and unpaired analyses with the Student's *t* test. Integrated responses to oral glucose were evaluated by analyses of area. Statistical analyses were performed by Professor Claude Cohen on the Northwestern University CDC-6400 Computer. The programs used were SPSS (Statistical Package for Social Scientists), BMDX70 (*t* test), and an integration routine to calculate areas via the method of overlapping parabolas.

RESULTS

1. *Basal Values after Overnight Fast:* Table 1 summarizes mean ± S.E.M. basal values for serum glucose and plasma IRI and glucagon after fourteen-hour overnight fast in sixteen subjects with normal carbohydrate metabolism and in ten subjects with gestational diabetes. Antepartum versus postpartum findings within each group have been analyzed on a pair-matched basis; the two groups have been compared at each time point by unpaired *t* test.

For glucose[2,5,6] and IRI,[1,2] our experiences in the sixteen normal subjects concorded with earlier reports: fasting glucose was reduced ($p < .01$) and IRI increased ($p < .001$) during late pregnancy in comparison to postpartum values (table 1). At the same time, their plasma immunoreactive glucagon was significantly greater antepartum than postpartum (59.6 ± 3.0 versus 43.2 ± 3.2 pg./ml.; $p < .001$). To interpret our glucagon findings, blood samples were secured following a fourteen-hour overnight fast from twenty-six age-matched nulliparous females with normal carbohydrate metabolism and negative family histories for diabetes. In these twenty-six subjects, mean ± S.E.M. values for serum glucose (88.0 ± 0.8 mg. per 100 ml.) and plasma IRI (6.0 ± 0.3 μU./ml.) were in the same range that we had observed in the normal subjects postpartum (table 1). However, levels of plasma immunoreactive glucagon (63.9 ± 5.8 pg./ml.) in these twenty-six control subjects were not significantly different from our antepartum values, and significantly ($p < .01$) exceeded the postpartum. Within this framework, it would appear that plasma glucagon after overnight fast does not deviate from nongravid values during the last trimester of pregnancy in subjects with normal carbohydrate metabolism, but that small, albeit significant, reductions in basal plasma glucagon may be seen five to eight weeks postpartum.

TABLE 1

Effects of Pregnancy on Basal Values for Serum Glucose and Plasma Immunoreactive Insulin (IRI) and Glucagon After Overnight Fast†

	n	Antepartum Glucose mg./100 ml.	Antepartum IRI μU./ml.	Antepartum Glucagon pg./ml.	Postpartum Glucose mg./100 ml.	Postpartum IRI μU./ml.	Postpartum Glucagon pg./ml.
Normal Carbohydrate Metabolism	(16)	78.1 ± 1.4	12.4 ± 1.1	60.1 ± 4.2	86.8 ± 1.4***	8.3 ± 0.6***	43.2 ± 3.3***
Gestational Diabetics	(10)	94.4 ± 4.8	21.3 ± 3.0	99.3 ± 19.9	98.6 ± 3.0	16.0 ± 3.4**	73.6 ± 18.8**
p (Normals vs. Gestational Diabetics)		< .01	< .05	< .10	< .01	< .05	N.S.

†Sixteen subjects with normal carbohydrate metabolism and ten gestational diabetics were examined antepartum and again postpartum as described in the text. Antepartum vs. postpartum values for the subjects within each group have been compared by paired *t* tests and statistically significant differences depicted by the asterisks next to the postpartum measurements (** denotes $p < .01$; *** denotes $p < .001$). Statistically significant differences between the two groups have been depicted by the p values at the bottom of the individual columns.

Somewhat different relationships were observed in the ten gestational diabetics (table 1). Their fasting serum glucose was not different antepartum than postpartum and exceeded values seen in the normal subjects at both time points even though none of these displayed fasting hyperglycemia. Plasma IRI was also increased in the gestational diabetics.* However, on paired analysis, in the gestational diabetics, as in the normals, fasting IRI was significantly greater antepartum than postpartum ($p < .01$). Fasting plasma glucagon followed a similar trend: antepartum values exceeded postpartum (table 1, $p < .01$). In absolute terms, the concentrations of plasma glucagon in the ten gestational diabetics antepartum (99.3 ± 19.9 pg./ml.) as well as postpartum (73.6 ± 18.8 pg./ml.) tended to be greater than in the sixteen subjects with normal carbohydrate metabolism although the differences between the two groups did not achieve statistical significance at either time point (table 1).

2. *Response to Oral Glucose:* The antepartum vs. postpartum changes in serum glucose, and plasma IRI and glucagon following 100 gm. glucose by mouth are compared on a pair-matched basis in the sixteen normal and ten gestational diabetics in figures 1 and 2 respectively. Results have been expressed on the basis of absolute increments or decrements from basal values and analyzed for statistical significance at each time point.

In the sixteen normal subjects (figure 1), the increments in glucose and IRI after oral glucose were significantly greater antepartum than postpartum from sixty minutes onward. Thus, when integrated over 180 minutes, net glucose area was approximately 35 per cent greater (102.0 ± 6.1 versus 75.0 ± 0.9 mg.-min./ml.; $p < .01$) and net insulin area was approximately 45 per cent greater (15,800 ± 1,850 versus 10,900 ± 1,230 μU.-min./ml.; $p < .01$) during pregnancy. In view of the known capacity for oral[11] or intravenous[12] glucose to suppress alpha-cell function, the concomitant changes in plasma glucagon were of particular interest. Plasma glucagon displayed small but significant reductions antepartum as well as postpartum within thirty minutes following glucose administration. However, coincident with the more marked and more prolonged hyperglycemia of pregnancy, the suppressions of plasma glucagon were greater antepartum than postpartum on a percentile as well as on an absolute basis (figure 1). Maximal decline of glucagon below basal values was 29.6 per cent during pregnancy and only 15.3 per cent postpartum.

Using USPHS criteria,[20] six of the ten gestational diabetics still exhibited borderline or definitely abnormal oral glucose tolerance postpartum. Nonetheless, for the group as a whole (figure 2), the antepartum vs. postpartum relationships for glucose and IRI were qualitatively similar to those seen in the nondiabetic group (figure 1). Thus, during the integrated 180-minute period following glucose administration, average glucose area was 39 per cent greater antepar-

*The precise basis for this is unclear. Conceivably, some of the higher IRI levels in the gestational diabetics could have been mediated via the higher values for fasting glucose. However, the gestational diabetics were also significantly heavier than the normal subjects (Mean ± S.E.M. for per cent of ideal body weight prior to pregnancy: 138.6 ± 9.8 versus 108.5 ± 3.6 per cent; $p < .01$) so that increased adiposity may have been the major determinant of the increased basal IRI.[19]

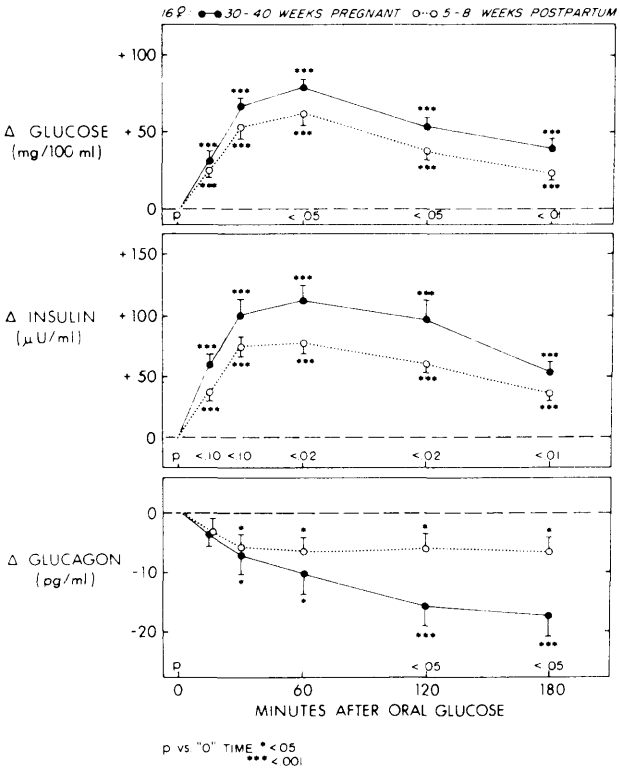

FIG. 1. Effects of pregnancy on the response of serum glucose and plasma insulin and glucagon to oral glucose in normal women. Oral glucose (100 gm.) was administered after overnight fast to sixteen normal women in weeks 30 to 40 of gestation and again five to eight weeks postpartum. Mean ± S.E.M. values for subsequent changes in glucose, insulin and glucagon are depicted. The asterisks denote significance of the changes at each time point (p versus "zero minutes": * = < .05, ** = < .01, and * = < .001) on the basis of paired comparison with fasting values in individual subjects. The p values at the bottom of each panel denote significance of differences between antepartum versus postpartum changes at each time point as estimated by paired *t* test.**

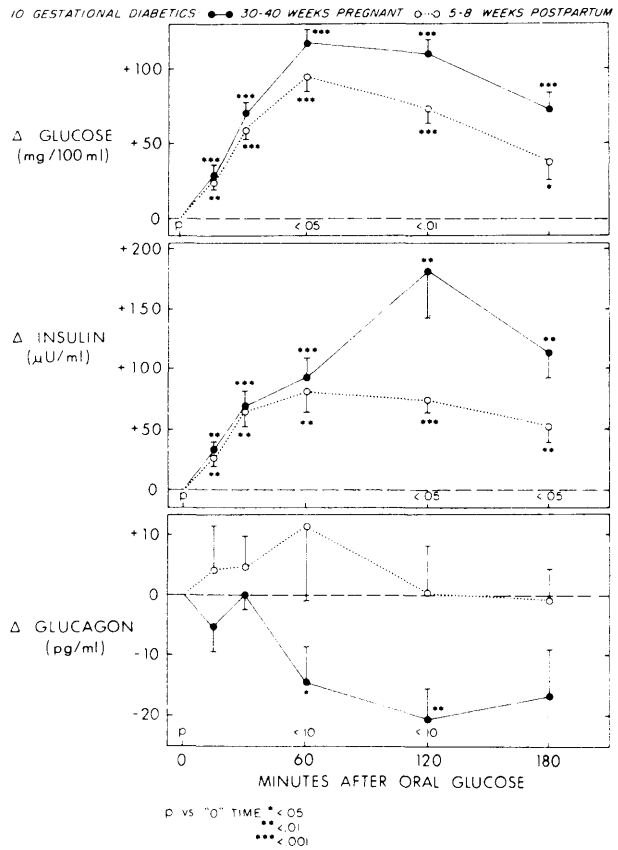

FIG. 2. Effects of pregnancy on the response of serum glucose and plasma insulin and glucagon to oral glucose in gestational diabetics. Oral glucose (100 gm.) was administered after overnight fast to ten gestational diabetics in weeks 30 to 40 of gestation and again five to eight weeks postpartum. Mean ± S.E.M. values for subsequent changes in glucose, insulin and glucagon have been presented and analyzed for statistical significance as in figure 1.

tum than postpartum (168 ± 12.0 versus 121 ± 12.7 mg.-min./ml., $p < .001$) and average insulin area was approximately 79 per cent greater (21,800 ± 4,450 versus 12,200 ± 1,780 μU.-min./ml., $p < .05$). However, the concomitant excursions in plasma glucagon deviated somewhat from the predicted pattern. In contrast to previous findings of alpha cell nonsuppressibility in established chemical diabetes,[11,12] the gestational diabetics displayed significant reductions in plasma glucagon at 60 ($p < .05$) and 120 ($p < .01$) minutes after oral glucose antepartum. The maximal decrements occurred at 120 minutes, coincident with the peak increases in plasma IRI and the persistent hyperglycemia. Conversely, postpartum, when glucose tolerance was improved and the late increases in plasma IRI were significantly smaller, the gestational diabetics did not exhibit significant reductions of plasma glucagon at any time point (figure 2). In this regard, their postpartum lack of glucagon suppressibility resembled the pattern previously reported in overt diabetes.[11,12]

DISCUSSION

By characterizing plasma glucagon after overnight fast and in response to oral glucose during late human pregnancy, the present studies have provided new insights about gestational glucoregulation. Subjects with normal carbohydrate metabolism and gestational diabetics were studied antepartum during week 30 to 40 of pregnancy, and again, postpartum five to eight

weeks following delivery. Such pair-matched observations in which each subject served as her own control, enabled us to draw conclusions from relatively small populations, and from differences in estimates of plasma glucagon which often bordered on the limits of analytical discrimination.

We have found that plasma glucagon after overnight fast in normal women in late pregnancy is the same as in age-matched, nongravid nullipara. Thus, hyperglucagonemia cannot be implicated in the manifestations of "accelerated starvation"[21] that are already evident at this time (such as the heightened mobilization of fat and the increased propensity to ketogenesis).[9,17] Moreover, it would appear that hyperglucagonemia does not constitute one of the beta-cytotropic or contrainsulin factors by which the basal hyperinsulinism and insulin-resistance of late pregnancy are mediated. Indeed, the association of a normal fasting plasma glucagon together with an increased plasma IRI which we observed is consistent with the prior histologic evidence for beta-cell preponderance in the islet hyperplasia of pregnancy.[8]

We cannot explain the small, but significant, reductions in basal plasma glucagon* that were seen five to eight weeks postpartum. Longitudinal studies of larger groups of postpartum women are underway to evaluate the duration of this phenomenon and its underlying determinants.

More dramatic effects of pregnancy on glucagon dynamics were seen when we evaluated the response to oral glucose. In our sixteen normal subjects, prompt decrements in plasma glucagon were observed antepartum as well as postpartum. However, the fall of circulating glucagon below basal values was significantly greater antepartum (on a relative basis, when expressed as a percentage, as well as in absolute terms when expressed in pg./ml.). The ante- versus postpartum differences were particularly pronounced at 120 and 180 minutes following glucose ingestion and coincided with the exaggerated hyperglycemia and activated secretion of insulin that are still demonstrable at this time as part of normal oral glucose tolerance in late pregnancy. The latter correlation suggests that in pregnancy, as under nongravid conditions, glucagon secretion may be subject to feedback suppression directly or indirectly by insulin-mediated glucose flux.[11,12,22] Thus, during late gestation, the more prolonged availability of increased amounts of glucose and insulin within the circulation could create a setting in which the alpha cells would be "turned off" more markedly following glucose ingestion.

Our findings in ten gestational diabetics are consistent with the above proposition. On paired comparison, significantly more intolerance to oral glucose was obtained during pregnancy so that hyperglycemia and attendant hyperinsulinemia were particularly more prolonged antepartum (figure 2). In association with these glucose-insulin relationships, plasma glucagon fell after oral glucose during pregnancy. Conversely, suppression of plasma glucagon was not demonstrable during the postpartum tests even though carbohydrate tolerance had improved. It could be postulated that diabetes is characterized by decreased sensitivity of both alpha and beta cells to metabolic signals. In mild diabetes, the beta cell is clearly less responsive but not absolutely refractory to the stimulatory effect of glucose.[23-26] In most of our present group of gestational diabetics, the alpha cells may also have been relatively insensitive to insulin-mediated glucose flux, as judged by the failure of glucose ingestion to suppress plasma glucagon postpartum. However, during gestation, the much higher and more prolonged excursions of plasma glucose and insulin may have masked (and overcome) this blunted responsiveness of the alpha cells. In any event, it is clear that nonsuppressibility of pancreatic alpha cells is not an initiating factor in that form of carbohydrate intolerance which has been designated "gestational diabetes."

On a more general note, the enhanced potential for alpha-cell suppression during late pregnancy may have certain metabolic implications. Elsewhere, we have suggested that there may be mechanisms to facilitate anabolism whenever the mother eats during late pregnancy and that these could compensate for the "accelerated starvation" that occurs when food is withheld.[27] Exaggerated suppression of plasma glucagon following glucose ingestion in late pregnancy could constitute one such form of "facilitated anabolism." It would *maximize* the glycogenic and lipogenic potential of the insulin secreted in response to glucose by *minimizing* the availability of glucagon for opposing glycogenolytic, gluconeogenic, and lipolytic actions.

*Inasmuch as measurements of glucagon made with 30K antiserum cannot be proven to be absolutely specific for glucagon, differences among groups of subjects (such as antepartum versus postpartum women) in the basal state could theoretically be the result of factors other than glucagon. Antiserum G-58 has been regarded as uninfluenced by factors other than glucagon. We therefore assayed thirty samples from nulliparous controls and antepartum as well as postpartum subjects with antisera G-58 and 30 K and found similar results with both. Thus, these two antisera, the most specific available within our laboratory, show antepartum versus postpartum differences to an equal degree.

However, it must be recognized that the alpha-cell response to other dietary components has not yet been established. Our reluctance to administer parenteral solutions for experimental purposes during human pregnancy has precluded direct evaluations with standardized alpha-cell secretogogues, such as intravenous alanine or arginine.[28,29] Hopefully, ongoing efforts with oral challenges (such as "mixed meals" or meat feedings) may provide these crucial additional characterizations.

ACKNOWLEDGMENT

The authors thank Drs. Henry J. Ruder and Richard L. Phelps for their help with the studies in some of the subjects and acknowledge the excellent technical assistance of Mary Kay Hilbrich Slocumb.

This study was supported in part by Research Grants AM-10699 and AM-02700, Training Grant AM-05071 from the National Institute of Arthritis and Metabolic Diseases, by Clinical Research Center Grant RR-48 from the Division of Research Resources, National Institutes of Health, Bethesda, Maryland, and by a grant from the Kroc Foundation.

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