HUMAN FETAL INSULIN SECRETION
IN RESPONSE TO MATERNAL GLUCOSE AND LEUCINE ADMINISTRATION.

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SUMMARY

Maternal and fetal serum insulin response to glucose, leucine and leucine plus glucose was examined by infusions to normal pregnant women at term immediately before cesarean section. The maternal infusion of glucose (50 g) for 30 or 60 minutes was associated with a marked hyperglycemia and with a rise in serum insulin in mother and fetus. The fetal insulin response to the administration of glucose for 60 minutes was higher ($p < 0.01$) than when the same dose was given for 30 minutes, while the blood glucose was lower ($p < 0.01$). The maternal infusion of a smaller dose of glucose (25 g) or leucine (15 g) for 60 minutes produced an increase in serum insulin only in the mothers. However the simultaneous administration of these substances stimulated maternal and fetal insulin secretion. The fetal insulin level produced was similar to that seen after the maternal infusion of 50 g of glucose for 60 minutes, while the cord blood glucose was slightly higher but significantly different ($p < 0.01$) than that seen in the saline infused group.

SPECULATION

It is suggested that amino acids with glucose regulate fetal insulin secretion.

Glucose rapidly crosses the placenta in the presence of a concentration gradient between mother and fetus and it has been suggested that fetal plasma glucose is controlled indirectly via the maternal endocrine system (3). Insulin is present in the fetal pancreas as early as the 10th gestational week and in fetal plasma from the 12th week (15, 17, 20). The insulin is of fetal origin since the human placenta is either impermeable or the amount transferred is physiologically insignificant (18). However, little information is available regarding the development of the mechanisms that control its secretion by the fetal beta cells or its physiological effects. Studies have demonstrated that early in gestation (12 to 20 weeks) fetal insulin response to maternal hyperglycemia is negligible (15). By term the fetus in utero is capable of responding to maternal hyperglycemia with an elevation of serum insulin, though the responses are attenuated compared with those of adults (15).

The object of the present study is to provide evidence that some amino acids, such as leucine, with glucose may contribute to the regulation of fetal insulin secretion. Twenty-five grams of glucose was infused with leucine to normal mothers just before cesarean section to increase cord blood glucose slightly. Blood glucose and serum insulin were determined in maternal blood during and after the infusion and in the fetus at birth. Furthermore, fetal cord blood glucose and serum insulin response to this maternal infusion were compared with those of the maternal administration of 50 g of glucose for 30 or 60 minutes.

METHODS

Sixty-one pregnant women who underwent cesarean section consented to participate in the study. Such sections were done for the following reasons: (1) previous section - 48 patients, (2) disproportion - one patient, (3) transverse lie - 4 patients, (4) advanced age of the mother - 8 patients. No patients were obese and none had a family history of diabetes mellitus or history of previous stillborn or large for date infants. They were chosen because the fetuses didn't undergo the stress of labor.

All studies were performed in the morning after 18-20 hours fast. A 20-gauge needle was placed in the right antecubital vein and one of the following substances was administered: (1) glucose 50 g or 25 g; (2) L-Leucine 15 g; (3) glucose 25 g plus L-Leucine 15 g; (4) saline.

Glucose 50 g was infused for 30, or 60 minutes while glucose, 25 g, L-Leucine and glucose plus L-Leucine were administered for 60 minutes.

The length of the infusion was synchronized so that birth occurred 10 minutes after it ended. Maternal venous blood samples were taken at 0 and every 30 minutes. Maternal venous and fetal umbilical mixed blood samples were obtained simultaneously at delivery.

All mothers received the premedication of atropine and promethazine. Anesthesia was induced by thiopental sodium and maintained by 50 per cent nitrous oxide and 50 per cent oxygen and succinylcholine.

Glucose was administered as a 10 per cent solution.

When glucose was mixed with L-Leucine, 50 ml of 50 per cent glucose was added to 750 ml of the amino acid solution.

Blood glucose was determined by a glucose oxidase method. Serum insulin was determined in triplicate by radioimmunoassay method (7).

RESULTS

Maternal infusion of glucose (50 g) for 30, 60 minutes and saline for 60 minutes on mother and fetus (Table I).

The fetal and maternal pancreas responded to glucose stimulus with an increase of insulin secretion.

At delivery after the administration of glucose to the mother for 30 minutes

the cord blood glucose and serum insulin were significantly higher than in the control group ($p < 0.05$).

The maternal infusion of the same amount of glucose for 60 minutes caused an increase of cord blood serum insulin significantly higher than seen when the same amount was infused for 30 minutes ($p < 0.01$), while the blood glucose was significantly lower ($p < 0.01$).

Maternal infusion of glucose (25 g), leucine (15 g), glucose (25 g) with leucine (15 g), or saline for 60 minutes on mother and fetus (Table II).

The maternal infusion of glucose caused a significant increase of serum insulin only in the mothers (maximum increase at 60 minutes). The fetal insulin was similar to that of the saline infused group, while the blood glucose was significantly increased ($p < 0.01$).

The maternal infusion of leucine caused a small but not significant rise of serum insulin only in the mother. The fetal insulin and blood glucose were not significantly different from those in the control group.

The maternal infusion of the glucose-leucine mixture caused an increase of maternal serum insulin with a maximum at 60 minutes. However, this increase was not significantly different from that observed at 60 minutes in the glucose infused group. In the fetus this mixture caused a marked increase of serum insulin which was significantly higher than in the control group ($p < 0.01$). The cord blood glucose was slightly higher but significantly different ($p < 0.01$) from that of the saline infused group.

DISCUSSION

As shown in Table I the fetus at term is capable of responding to maternal hyperglycemia with an elevation of serum insulin. After the maternal infusion of glucose for 30 minutes insulin was significantly higher than in the control group. However, from the table it can be observed that the duration rather than the degree of hyperglycemia determined the fetal insulin response. In fact when glucose was given to the mother for 60 minutes, the fetal insulin response was higher than seen when the same amount of glucose was infused for 30 minutes.

The reason for this phenomenon may be related to a slow insulinogenic response of the fetal pancreas to glucose. Milner and Hales (11) studied maternal and fetal insulin response to intravenous glucose given rapidly between 4 and 100 minutes before delivery and found that the fetal plasma insulin levels increased with time up to one hour. The acute insulin secretory response to the increase in blood glucose concentration, which is characteristic in the adult, was absent. Subsequently similar observation were made by Tobin et al. (21), Cordero et al. (2) and Oakley et al. (14) using the Saling technique. On the other hand Coltart et al. (1) showed that the rapid infusion of glucose to normal women before the onset of labour caused a rise in insulin five minutes after the glucose injection in four fetuses, in one fetus a gradual rise which reached the maximum at 70 minutes, and in the remaining three fetuses no rise was seen. Even if these studies demonstrated fetal insulin secretion after maternal infusion of glucose, it can be noted that the cord blood glucose levels were abnormally elevated. In fact, the normal fetus lives in an environment where blood glucose levels fluctuate little and are under maternal control. In normal pregnant women plasma glucose remains remarkably constant over the 24 hour period, rarely exceeding 100 mg/dl except immediately after eating, while the concentration of fetal blood glucose mirrors that of the mother and is slightly lower (7). Our data along with the above mentioned studies (2, 11, 14, 21) confirm the concept postulated by Pedersen (16) that the hyperinsulinemia of the infant of diabetic mother is due to maternal and consequent fetal hyperglycemia.

Contrastingly, the findings summarized in Table II show that other nutrients such as leucine with glucose may contribute in modulating the fetal insulin secretion in the presence of moderate hyperglycemia. The simultaneous administration of glucose (25 g) with leucine (15 g) for 60 minutes caused a marked increase of cord blood serum insulin. The serum insulin level produced was similar to that seen after the maternal infusion of 50 g of glucose for 60 minutes, while the cord blood glucose level was only slightly higher than that seen in the control group. Separate infusion on these substances produced a small increase in serum insulin only in the mothers. These observations support our previous data that leucine (15 g) with glucose (50 g) administered to the mother stimulated maternal and fetal insulin secretion (8). However, this infusion administered to the mother for 30 minutes caused an excessive maternal and fetal hyperglycemia.

The data concerning the role of amino acids in insulin secretion in the fetus are limited and often discordant. It has been reported that isolated human fetal pancreatic tissue releases insulin when incubated with leucine or arginine but not with glucose (13, 19). Fujimoto and Williams (6) showed that when glucose was used alone or with arginine it failed to stimulate insulin secretion in organ culture of pancreas from human fetuses between 8 and 20 weeks gestation. Glucose becomes a potent stimulus in the presence of methylxanthines or of glucagon (6, 12). In vivo, both during early pregnancy and at term, the human fetus failed to respond to maternal arginine infusion with an insulin rise (10). In our study also the maternal infusion of leucine caused no change in fetal serum insulin. In order to explain the lack of fetal serum insulin response to amino acids in vivo it has been suggested that their blood glucose concentrations are lower than adults (10). In fact the insulinogenic effect of these substances in adults depends upon a normal blood glucose concentration and is enhanced by hyperglycemia (4). In the present study the simultaneous administration of glucose and leucine caused a marked increase of fetal insulin secretion. However, an unexpected finding seen by us was that the synergistic effect was not evident in the mothers. A possible explanation is that the slow infusion rate of leucine and its transfer to fetus through the placenta caused a low maternal leucine concentration.

CONCLUSION

It has been proposed that normal fetal development and growth depend upon aminoacid transport and protein synthesis which are insulin dependent whereas glucose homeostasis is achieved by maternal control (3). Amino acids are actively transferred from the maternal to the fetal circulation and their concentration in the fetal blood are higher than in maternal blood (22). Whether rising levels of amino acids and glucose, such as after a mixed protein-carbohydrate meal, may stimulate fetal insulin secretion, is unknown.

REFERENCES AND NOTES

- Coltart, T.M., Beard, R.W., Turner, R.C., and Oakley, N.W.: Blood glucose and insulin relationships in the human mother and fetus before onset of labour. *Brit. Med. J.*, 4: 17 (1969).
- Cordero, L., Jr., Grunt, J.A., and Anderson, G.G.: Hypertonic glucose infusion during labor. Maternal-fetal serum insulin relationships. *Amer. J. Obstet. Gynecol.*, 107: 560 (1970).
- Cornblath, M. and Schwartz, R.: Disorders of carbohydrate metabolism in infancy, p.29 (W.B. Saunders Company, Philadelphia, London, Toronto 1976).
- Efendic, S., Cerasi, E., and Luft, R.: Role of glucose in arginine-induced insulin release in man. *Metabolism*, 20: 568 (1971).
- Espinosa de los Monteros, M.A., Driscoll, S.G., and Steinke, J.: Insulin release from isolated human fetal pancreatic islets. *Science*, 168: 1111 (1970).
- Fujimoto, W.Y., and Williams, R.H.: Insulin release from cultured fetal human pancreas. *Endocrinology*, 91: 1133 (1972).
- Gilmer, M.D.G., Beard, R.W., Brooke, F.M., and Oakley, N.W.: Carbohydrate metabolism in pregnancy. Part I. Diurnal plasma glucose profile in normal and diabetic women. *Brit. Med. J.*, 3: 399 (1975).
- Grasso, S., Palumbo, G., Messina, A., Mazzarino, C., and Reitano, G.: Human maternal and fetal serum insulin and growth hormone (HGH) response to glucose and leucine. *Diabetes*, 25: 545 (1976).
- Hales, C.N., and Randle, P.J.: Immunoassay of insulin with insulin antibody precipitate. *Biochem. J.*, 88: 137 (1963).
- King, K.C., Butt, J., Raivio, K., RWihM, N., Roux, J., Teramo, K., Yamaguchi, K., and Schwartz, R.: Human maternal and fetal insulin response to arginine. *New Engl. J. Med.*, 285: 607 (1971).
- Milner, R.D.G., and Hales, C.N.: Effect of intravenous glucose on concentration of insulin in maternal and umbilical-cord plasma. *Brit. Med. J.*, 1: 284 (1965).
- Milner, R.D.G., Barson, A.J., and Ashworth, M.A.: Human foetal pancreatic insulin secretion in response to ionic and other stimuli. *J. Endocrinol.*, 51: 323 (1971).
- Milner, R.D.G., Ashworth, M.A., and Barson, A.J.: Insulin release from human foetal pancreas in response to glucose, leucine and arginine. *J. Endocrinol.*, 52: 497 (1972).
- Oakley, N.W., Beard, R.W., and Turner, R.C.: Effect of sustained maternal hyperglycemia on the fetus in normal and diabetic pregnancies. *Brit. Med. J.*, 1: 466 (1972).
- Obenshain, S.S., Adam, P.A.J., King, K.C., Teramo, K., Raivio, K.O., Raiha, N., and Schwartz, R.: Human fetal insulin response to sustained maternal hyperglycemia. *New Engl. J. Med.*, 283: 566 (1970).
- Pedersen, J.: Weight and length at birth of infants of diabetic mothers. *Acta Endocrinol.*, 16: 330 (1954).
- Rastogi, G.K., Letarte, J., and Fraser, T.R.: Immunoreactive insulin content of 203 pancreases from fetuses of healthy mothers. *Diabetologia*, 6: 445 (1970).
- Sabata, V., Frerichs, H., Wolf, H., and Stubbe, P.: Insulin and glucose levels in umbilical cord blood after infusions of glucose with insulin to women in labour. *J. Obstet. Gynaecol.*, 77: 121 (1970).
- Schaeffer, L.D., Wilder, M.L., and Williams, R.H.: Secretion and content of insulin and glucagon in human fetal pancreas slices in vitro. *Proc. Soc. Exp. Biol. Med.*, 143: 314 (1973).
- Steinke, J., and Driscoll, S.G.: The extractable insulin content of pancreases from fetuses and infants of diabetic and control mothers. *Diabetes*, 14: 573 (1965).
- Tobin, J.D., Roux, J.F., and Soeldner, J.S.: Human fetal insulin response after acute maternal glucose administration during labor. *Pediatrics*, 44: 668 (1969).
- Young, M., and Prenton, M.A.: Maternal and fetal plasma aminoacid concentrations during gestation and in retarded fetal growth. *J. Obstet. Gynaecol. Brit. Cwlth.*, 76: 333 (1969).
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Table I

Comparison of maternal and fetal blood glucose and serum insulin (Mean±SEM) in healthy pregnant women at term infused for 30 or 60 minutes with glucose (50 g) or with saline for 60 minutes.

	MOTHER				U C(2)
	0'	30'	40'	Delivery	
MATERNAL INFUSION OF GLUCOSE FOR 30 MIN					n = 7
b w (1)	3177 ± 165 g				
Blood Glucose (mg/dl)	79±2	306±5	282±13	230±5	
Serum Insulin (μU/ml)	23±5	76±9	52±9	32±4	
	MOTHER				U C(2)
	0'	30'	60'	70'	
MATERNAL INFUSION OF GLUCOSE FOR 60 MIN					n = 10
b w (1)	3405 ± 95 g				
Blood Glucose (mg/dl)	69±3	157±11	186±13	171±14	132±9
Serum Insulin (μU/ml)	18±3	83±13	94±14	89±10	61±5
MATERNAL INFUSION OF SALINE FOR 60 MIN					n = 6
b w (1)	3308 ± 159 g				
Blood Glucose (mg/dl)	74±4	79±5	84±5	84±7	64±5
Serum Insulin (μU/ml)	20±3	22±6	24±5	22±5	18±3

- (1) b w : body weight of the infants.
(2) U C : umbilical cord.

Table II

Comparison of maternal and fetal blood glucose and serum insulin (Mean±SEM) in healthy pregnant women at term infused for 60 minutes with saline, glucose, leucine or glucose in combination with leucine.

	MOTHER				U C (2)
	0'	30'	60'	70'	
	Delivery				
MATERNAL INFUSION OF GLUCOSE (25 g)					n =11
b w (1)	3768 $\pm$ 64 g				
Blood Glucose (mg/dl)	64 $\pm$ 1	106 $\pm$ 6	122 $\pm$ 6	106 $\pm$ 4	82 $\pm$ 3
Serum Insulin (μ U/ml)	14 $\pm$ 1	48 $\pm$ 8	78 $\pm$ 17	33 $\pm$ 7	21 $\pm$ 3
MATERNAL INFUSION OF LEUCINE (15 g)					n =8
b w (1)	3300 $\pm$ 178 g				
Blood Glucose (mg/dl)	69 $\pm$ 2	72 $\pm$ 4	74 $\pm$ 4	77 $\pm$ 4	70 $\pm$ 6
Serum Insulin (μ U/ml)	13 $\pm$ 2	27 $\pm$ 6	28 $\pm$ 8	21 $\pm$ 5	14 $\pm$ 1
MATERNAL INFUSION OF GLUCOSE (25 g) WITH LEUCINE (15 g)					n =9
b w (1)	3605 $\pm$ 130 g				
Blood Glucose (mg/dl)	75 $\pm$ 3	111 $\pm$ 7	124 $\pm$ 7	115 $\pm$ 7	86 $\pm$ 3
Serum Insulin (μ U/ml)	19 $\pm$ 4	64 $\pm$ 10	95 $\pm$ 14	71 $\pm$ 11	64 $\pm$ 8
MATERNAL INFUSION OF SALINE					n =10
b w (1)	3340 $\pm$ 119 g				
Blood Glucose (mg/dl)	76 $\pm$ 3	80 $\pm$ 4	84 $\pm$ 5	90 $\pm$ 5	65 $\pm$ 4
Serum Insulin (μ U/ml)	25 $\pm$ 2	25 $\pm$ 3	28 $\pm$ 3	22 $\pm$ 3	19 $\pm$ 3

- (1) b w : body weight of the infants.
(2) U C : umbilical cord.