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Very Immature Infants (≤ 30 Wk) Respond to Glucose Infusion with Incomplete Suppression of Glucose Production

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ABSTRACT

Small substrate stores and immature enzyme systems make preterm infants prone to develop hypoglycemia. Hyperglycemia may also occur, particularly when these infants are given i.v. glucose. To evaluate the capacity for regulation of glucose production in response to glucose infusion, 10 newborn infants, born after 25–30 wk of gestation, were studied. Their glucose production rates (GPR) were calculated and the concentrations of glucose, insulin, and glucagon in plasma were measured during infusion of glucose at a rate of first 1.7 ± 0.2 and then 6.5 ± 0.3 $mg \cdot kg^{-1} \cdot min^{-1}$ (9.4 ± 1.1 and 36.1 ± 1.7 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) (mean ± SD). GPR was determined by use of D-6,6-$^2H_2$-glucose. When the rate of infusion of glucose was increased, GPR decreased from 4.3 ± 1.3 to 1.4 ± 1.1 $mg \cdot kg^{-1} \cdot min^{-1}$ (23.9 ± 7.2 to 7.8 ± 6.1 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) (mean ± SD) ($p = 0.00006$). In addition, the plasma insulin concentration increased from 6 ± 2 to 11 ± 4 $\mu U \cdot mL^{-1}$ ($p = 0.006$) and the plasma glucose concentration from 3.6 ± 1.1 to 6.1 ± 1.3 mM (mean ± SD) ($p = 0.0002$), whereas the glucagon concentration remained unchanged. Only the insulin concentration in plasma was significantly related to GPR. The results show that very immature newborn infants have an incomplete and varying capacity to respond to glucose infusion with suppression of glucose production. Insulin seems to be more important than plasma glucose in the regulation of glucose homeostasis in these infants. (*Pediatr Res* 36: 550–555, 1994)

Abbreviations

CV, coefficient of variation
GPR, glucose production rate

At birth the transplacental supply of nutrients to the fetus is interrupted and the newborn infant has to mobilize fuel for the metabolism of vital organs, especially the CNS. As a result of defective glycogenolysis and gluconeogenesis, this adaptation is sometimes incomplete, particularly in immature infants (1). Their substrate stores are limited and rapidly depleted, frequently resulting in hypoglycemia (2). The prevention and treatment of hypoglycemia necessitates infusion of glucose to supplement the small amounts of oral feeding tolerated by these infants. Even during i.v. infusions of glucose at a low rate, hyperglycemia is a commonly observed complication in very immature infants, indicating defective glucose regulation (3, 4). In adults and experimental animals, the glucose production is completely suppressed when glucose is given at a rate corresponding to the basal hepatic output (5–8). The adult control of glucose homeostasis is mediated by increased insulin secretion and by a direct effect of glucose on the hepatic glucose production (9, 10). Very little information is available concerning the capacity for glucose regulation in immature newborns. As in adults, the glucose homeostasis may be controlled by the plasma insulin, glucagon, and glucose concentration, but other factors such as stress and insulin resistance may also be of importance (6, 7, 11). The high brain/body weight ratio in newborn infants probably also influences the glucose turnover (12). Glucose regulation during infusion of glucose has previously been studied in a few term and slightly preterm newborn infants by use of compounds labeled with stable isotopes (4, 13–15). In these studies, a variable degree of suppression of glucose production was found.

The main purpose of the present study was to determine whether very immature infants are capable of decreasing their glucose production in response to an i.v. infusion of glucose given at a rate corresponding to the basal hepatic glucose output (12, 16–18). An additional aim was to investigate the influence of the plasma insulin, glucagon, and glucose concentrations on observed changes in the glucose turnover.

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METHODS

Subjects. The study comprised 10 newborn infants, born after 25–30 completed wk of gestation (Table 1). All were delivered at the University Hospital in Uppsala and admitted to the neonatal intensive care unit. The study was approved by the Human Ethics Committee of the Medical Faculty of the University of Uppsala. Parental consent was obtained after oral and written information was given.

The infants of the study group were carefully selected to represent "healthy" infants with healthy mothers and uncomplicated deliveries. The gestational age of the infants was estimated from the maternal menstrual history and was confirmed by routine ultrasound examination during pregnancy and by physical characteristics according to the method of Finnström (19). The birth weights of the infants ranged from 630 to 1430 g, which is within ±2 SD of the Swedish growth chart. Five of them were delivered by cesarean section (nos. 1, 2, 3, 6, and 9). None of the infants were asphyxiated (Apgar score <7 at 5 min). The infants were nursed in incubators with a relative ambient humidity of 85–90%. The infants were clinically stable. One of the infants (no. 8) needed intermittent positive-pressure ventilation because of infant respiratory distress syndrome. Eight of them had a mild respiratory disease, requiring treatment with continuous positive airway pressure. One infant (no. 3) needed no ventilatory assistance at all. All of the infants were normoventilated, had a normal pH and body temperature (36.5–37°C), and were normoxemic, seven of 10 on air and three of 10 with an oxygen supply of ≤30%. Aminophylline (Teofyllamin, Kabi Vitrum, Stockholm, Sweden) (6 $mg \cdot kg^{-1} \cdot d^{-1}$) was given as apnea prophylaxis to seven of the infants (nos. 1, 4, 5, 6, 7, 9, and 10).

The infants were studied at a postnatal age of between 21 and 88 h (Table 1). All of them were given glucose i.v. directly after birth and small amounts of oral feeding from the age of 2 h. The total volume given (i.v. and oral) was 65 $mL \cdot kg^{-1} \cdot d^{-1}$ on d 1, 75 $mL \cdot kg^{-1} \cdot d^{-1}$ on d 2, 90 $mL \cdot kg^{-1} \cdot d^{-1}$ on d 3, and 100 $mL \cdot kg^{-1} \cdot d^{-1}$ on d 4 postnatally. The total amount of breast milk given during the last 24 h before the study ranged from 0 to 59 $mL \cdot kg^{-1}$, corresponding to 2.7 ± 1.8 $mL \cdot kg^{-1}$ (range 0–5.6 $mL \cdot kg^{-1}$) every second hour. Because at least one meal was excluded before the start of the study, the period without oral feeding before the first sampling period ranged from 3 to 25 h with a mean (± SD) of 6.7 ± 6.5 h. The mean glucose infusion rate (± SD) at the start of the study was 5.0 ± 1.7 $mg \cdot kg^{-1} \cdot min^{-1}$ (27.8 ± 9.4 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) (Table 2).

Table 1. *Patient characteristics*

Patient no.	Gestational age (wk)	Birth weight (g)	Actual weight (g)	Postnatal age (h)
1	26	1015	909	71
2	27	1257	1278	21
3	30	1430	1185	87
4	28	1420	1288	88
5	27	1242	1117	60
6	26	951	806	69
7	25	640	540	52
8	25	630	604	52
9	28	1250	1049	77
10	28	1081	989	44
Mean	27	1092	976	62
SD	2	287	262	21

Isotope tracer. The tracer used was D-6,6-$^2H_2$-glucose (isotopic purity 98 atom %), which was purchased from Cambridge Isotope Laboratories, Woburn, MA. The deuterated glucose, dissolved in 0.9% saline in a concentration of 4.5 $mg \cdot mL^{-1}$, was sterile in microbiologic cultures and pyrogen-free when tested by the Limulus lysate method (20).

Study design. After obtaining a baseline blood sample, the tracer was administered as a priming dose of 5 $mg \cdot kg^{-1}$ (27.8 $\mu mol \cdot kg^{-1}$) (given over a 10-min period) followed by an infusion given at a constant rate of 0.19 ± 0.11 $mg \cdot kg^{-1} \cdot min^{-1}$ (1.06 ± 0.61 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) (mean ± SD) for 160 min in eight patients and for 180 min in two patients (nos. 1 and 2). At the start of the study, the infusion rate of unlabeled glucose was decreased from 5.0 ± 1.7 $mg \cdot kg^{-1} \cdot min^{-1}$ (27.8 ± 9.4 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) (mean ± SD) to 1.7 ± 0.2 $mg \cdot kg^{-1} \cdot min^{-1}$ (9.4 ± 1.1 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) (mean ± SD) and was given at this rate during the first 80 min. During the remaining 80–100 min of the study period, the infusion rate was increased to 6.5 ± 0.3 $mg \cdot kg^{-1} \cdot min^{-1}$ (36.1 ± 1.7 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) (mean ± SD), approximately corresponding to basal hepatic output (12, 16, 17, 18). The isotopic compound and the unlabeled glucose were administered in a peripheral vein with calibrated volumetric pumps (IMED 965 micro, IMED, Oxford, UK). At each infusion rate, the last 20 min constituted the sampling period, during which three blood samples (250 μL) were obtained at 10-min intervals. No episodes of hypoglycemia (plasma glucose ≤ 1.6 mM) occurred. The blood samples were collected into ice-cold EDTA tubes from another peripheral vein in patients 3, 4, 5, 6, and 9 and from umbilical artery catheters, which were inserted for clinical purposes, in patients 1, 2, 7, 8, and 10. To avoid contamination with glucose, catheters used for blood sampling were rinsed and infused with saline throughout the entire study period.

Preparation and analysis of blood samples for determination of glucose concentration and isotopic enrichment. Plasma was immediately separated by centrifugation and the plasma glucose concentration was measured within 5 min by the glucose oxidase-peroxidase method in a glucose analyzer (Ames Minilab 1, Bayer AG, Leverkusen, Germany) as described earlier (16). The remaining plasma was frozen at −70°C pending further analysis. For studies of isotopic enrichment, the plasma samples were deproteinized with perchloric acid and then subjected to anion/cation exchange chromatography and derivatization with butaneboronic acid and acetic anhydride (16, 21, 22). The isotopic enrichment of dideuteroglucose in

Table 2. *Rate of infusion of unlabeled glucose at start of study and during steady state periods, amount of oral feeding per kg during last 24 h before study, plasma glucose concentration at start of study and during steady state periods, GPR during steady state periods, and change in GPR when glucose infusion rate was increased**

Patient no.	Glucose infusion rate ($mg \cdot kg^{-1} \cdot min^{-1}$)†			Oral feeding (mL/kg)	Plasma glucose (mM)†			GPR ($mg \cdot kg^{-1} \cdot min^{-1}$)†		Change in GPR (%)
	Start	I	II		Start	I	II	I	II	
1	5.5	1.7	6.4	20	4.3	3.1	5.2	3.4	0.2	−94
2	3.9	1.3	5.9	5	8.2	2.9	6.5	2.8	1.6	−43
3	2.8	1.7	6.6	59	3.2	2.4	4.5	3.2	0.4	−88
4	2.3	1.7	6.7	54	3.7	3.6	5.1	3.4	0.5	−85
5	6.7	1.8	6.7	30	6.6	3.0	6.5	5.1	2.1	−59
6	4.1	1.7	6.6	42	4.5	3.7	6.7	4.5	0.5	−89
7	6.2	1.9	6.8	19	6.4	4.0	5.4	4.9	0.7	−86
8	5.5	1.7	6.6	33	7.1	6.1	9.0	7.2	1.8	−75
9	7.9	1.6	6.4	0	5.3	2.6	5.3	3.2	3.8	19
10	5.1	1.7	6.7	9	6.6	4.5	7.0	4.9	2.1	−57
Mean	5.0	1.7	6.5	27	5.6	3.6	6.1	4.3	1.4	−66
SD	1.7	0.2	0.3	20	1.6	1.1	1.3	1.3	1.1	34

* For glucose, 1 μmol = 0.18 mg.

† I represents the first steady state period; II represents the second steady state period.

plasma was measured by gas chromatography/mass spectrometry in the electron impact mode (70 eV), using a Finnigan SSQ 70 mass spectrometer (Finnigan MAT, San José, CA) equipped with a Varian gas chromatograph (Varian Associates Inc., Sunnyvale, CA) with a DB-17 capillary column (30 m × 0.32 mm). The M-57 ion (m/z 297) and the corresponding M+2 ion (m/z 299), reflecting unlabeled and labeled glucose, respectively, were monitored selectively (21, 22).

Calculations. Isotopic enrichment (given as labeled/unlabeled glucose) was calculated from a standard curve obtained by gradually increasing dideuteroglucose in relation to unlabeled glucose. During the sampling periods, approximate steady state (23) was attained with regard to isotopic enrichment and glucose concentration in plasma. GPR was calculated during these periods. The 95% confidence interval for the CV regarding isotopic enrichment during infusion of glucose at the low rate was 7.6 ± 2.5%, and that during infusion at the high rate 5.9 ± 1.8% (Table 3). The corresponding figures for the plasma glucose concentrations were 4.4 ± 1.7% and 5.1 ± 1.4%, respectively. GPR was calculated as follows: GPR = infusion rate of deuterated glucose ($mg \cdot kg^{-1} \cdot min^{-1}$)/isotopic enrichment in plasma (%) − infused amount of unlabeled glucose ($mg \cdot kg^{-1} \cdot min^{-1}$). Isotopic enrichment is given as labeled/unlabeled glucose (%) (16). Because the length of time without oral feeding was at least 3 h before the first steady state period and the amount of breast milk given was small, the enterohepatic contribution of glucose was assumed to be marginal and has not been taken into account in the calculations.

Table 3. *CV during steady state periods with regard to plasma glucose concentrations and isotopic enrichments*

Patient no.	Plasma glucose		Isotopic enrichment	
	CV% plateau I	CV% plateau II	CV% plateau I	CV% plateau II
1	6.5	4.4	3.6	4.2
2	4.7	8.3	3.6	12.4
3	8.6	3.4	5.8	5.5
4	8.8	3.0	11.4	5.5
5	3.3	6.4	8.7	5.2
6	1.5	8.4	10.0	6.0
7	2.9	3.2	9.9	2.0
8	2.5	4.4	14.2	5.0
9	3.8	7.6	2.0	7.1
10	4.4	2.2	7.0	5.4

The plasma insulin concentration was measured by RIA using an antibody against porcine insulin raised in guinea pigs, human insulin standard, and ^{125}I-labeled human insulin (NOVO, Copenhagen, Denmark). The sensitivity of the assay was 0.2 μU per tube at a sample volume of 50 μL. The intraassay CV was 5.1% and the interassay CV 9.7%. Glucagon was measured by RIA (24) using 30K antibodies (Roger Unger, Dallas, TX), porcine glucagon standard, and ^{125}I-labeled porcine glucagon (NOVO). The sensitivity was 5 pg per tube at a sample volume of 50 μL. The intraassay CV was 10.0% and the interassay CV 13.8%. Because of the small plasma volumes available, portions of plasma from the three samples obtained during each of the two steady state periods were pooled. In one of the infants (no. 1), the amount of plasma available was insufficient for measurement of insulin and glucagon.

Statistical analysis. Simple linear regression analysis was used to test for statistical significance of variables related to the suppression of glucose production, whereas a multiple regression analysis was used to test the combined effect of insulin and glucose. A paired *t* test was used to test for statistical significance when variables obtained during the two infusion periods were compared. A p value of <0.05 was considered statistically significant.

RESULTS

The plasma glucose concentration at the start of the study was 5.6 ± 1.6 mM (mean ± SD). After infusion of

glucose at the low rate (1.7 ± 0.2 mg·kg^{-1}·min^{-1}) (9.4 ± 1.1 μmol·kg^{-1}·min^{-1}), this concentration had decreased (p = 0.005) to 3.6 ± 1.1 mM (mean ± SD) in the first sampling period (Fig. 1). A new plateau with an increased plasma glucose concentration of 6.1 ± 1.3 mM (mean ± SD) (p = 0.0002) had been attained 1 h after infusion of glucose at the high rate (6.5 ± 0.3 mg·kg^{-1}·min^{-1}) (36.1 ± 1.7 μmol·kg^{-1}·min^{-1}) (Fig. 1.) The plasma glucose concentration during the second sampling period did not differ significantly from that at the start of the study, although the rate of infusion of glucose was higher (p = 0.013) during this period compared with that before the study period (6.5 ± 0.3 and 5.0 ± 1.7 mg·kg^{-1}·min^{-1}) (36.1 ± 1.7 and 27.8 ± 9.4 μmol·kg^{-1}·min^{-1}) (mean ± SD). GPR decreased from 4.3 ± 1.3 mg·kg^{-1}·min^{-1} (23.9 ± 7.2 μmol·kg^{-1}min^{-1}) (mean ± SD) during infusion of glucose at the low rate to 1.4 ± 1.1 mg·kg^{-1}·min^{-1} (7.8 ± 6.1 μmol·kg^{-1}·min^{-1}) (mean ± SD) (p = 0.00006) during infusion at the high rate (Table 2, Fig. 2).

The change in GPR did not correlate with plasma glucose concentration at the start of the study, increase in plasma glucose or peak plasma glucose concentration, or the plasma glucose concentration during the steady state periods. Neither was this change related to birth weight, gestational age, postnatal age, length of time without oral feeding before the study, or the total amount of oral feeding or glucose given during the last 24 h before the study. The plasma insulin concentration at the low-rate infusion of glucose was 6 ± 2 μU·mL^{-1} (mean ± SD). When glucose was infused at the high rate, this concentration increased to 11 ± 4 μU·mL^{-1} (mean ± SD) (p = 0.006) (Table 4). The insulin concentration in plasma was negatively correlated with GPR (p = 0.011, r = −0.59) (Fig. 3), but was not correlated with the plasma glucose concentration. Glucose clearance and rate of appearance and disappearance of glucose were calculated and correlated with insulin, concentration of glucose in plasma, and glucose infusion rate. Furthermore, multiple regression analysis was performed to test the combined effect of insulin and glucose on GPR. None of these calculations did reveal any additional information. There was a large variation in the plasma concentrations of glucagon

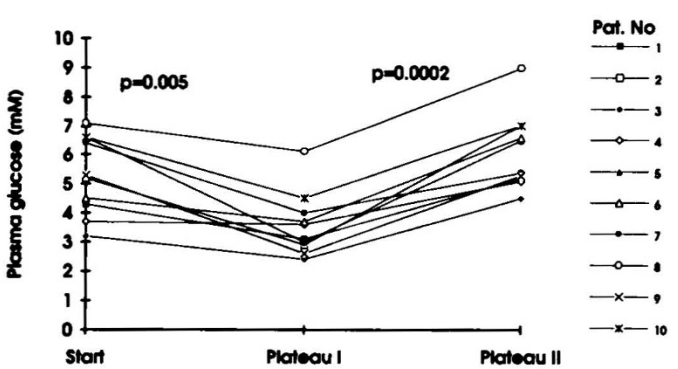

Figure 1. Plasma glucose concentration at the start of the study and during the sampling periods. *Plateau I* represents glucose infusion at a mean rate of 1.7 and *Plateau II* at a mean rate of 6.5 mg·kg^{-1}·min^{-1}. (For glucose, 1 μmol = 0.18 mg.)

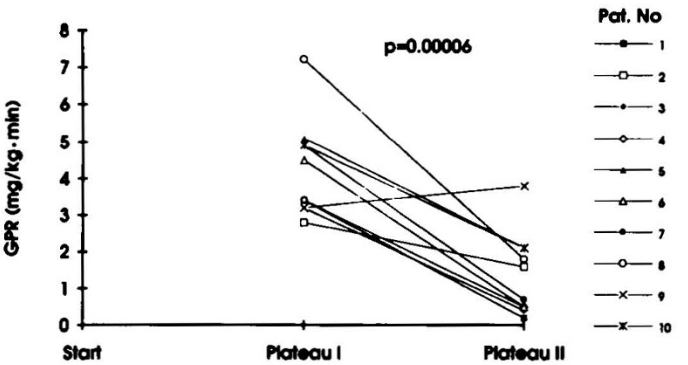

Figure 2. GPR during the sampling periods. *Plateau I* represents glucose infusion at a mean rate of 1.7 and *Plateau II* at a mean rate of 6.5 mg·kg^{-1}·min^{-1}. (For glucose, 1 μmol = 0.18 mg.)

(Table 4), and no significant differences were found between the sampling periods. There was no correlation between glucagon concentration and GPR.

DISCUSSION

The present results show that very immature infants (25–30 wk) respond to a glucose infusion, given at a rate corresponding to the basal hepatic glucose output, with incomplete suppression of their glucose production. There was a large variation between the individual infants in accordance with reports by Cowett *et al.* (5, 14) and Kalhan *et al.* (13) in studies of term and slightly preterm infants. Kalhan *et al.* (13) found that the GPR was negatively correlated to the peak plasma glucose concentration but not to the insulin concentration, in spite of an increased insulin secretion in response to glucose infusion. The results suggested that in the presence of insulin the plasma glucose concentration is the major regulatory factor for glucose production. Cowett *et al.* (5, 14) reported both increased plasma insulin concentration in response to a glucose infusion and a significant negative relationship between the insulin concentration and GPR. In contrast to Kalhan *et al.* (13), they found no correlation between GPR and the plasma glucose concentration.

Table 4. *Insulin and glucagon concentration in plasma during infusion of glucose at a mean rate of 1.7 (plateau I) and of 6.5 mg·kg^{-1}·min^{-1} (plateau II)*

Patient no.	Insulin I* (μU/mL)	Insulin II† (μU/mL)	Glucagon I* (pg/mL)	Glucagon II† (pg/mL)
1				
2	5	9	212	134
3	4	9	328	378
4	11	20	196	140
5	5	15	125	112
6	4	12	287	210
7	4	9	80	263
8	7	9	539	407
9	6	7	88	85
10	7	9	55	82
Mean	6	11	212	201
SD	2	4	155	121

* Plateau I.
† Plateau II.

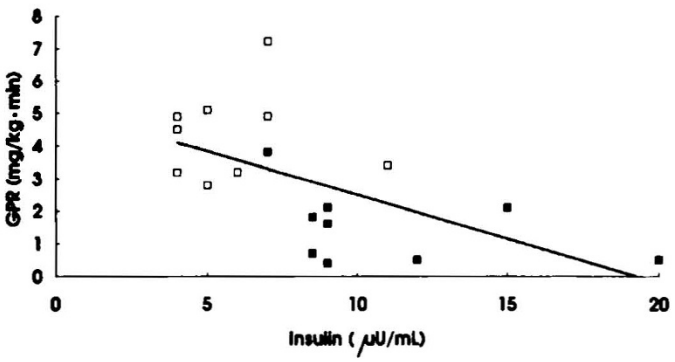

Figure 3. Linear regression between plasma insulin concentration and GPR. □, Plateau I; ■, Plateau II.

On the basis of these results, it was concluded that insulin was the most important factor in the regulation of the glucose production during infusion of glucose (5, 14). Similar to Cowett *et al.* (5, 14), we found that GPR was negatively correlated with the plasma insulin but not with the plasma glucose concentration. The insulin levels were lower than those reported by Cowett *et al.* (5, 14), but there was a corresponding 2-fold increase during the high-rate infusion of glucose even in our very immature infants. Even though insulin seems to be more important than plasma glucose in the regulation of glucose production, the insulin response of the infants was probably too low to achieve a more complete suppression of the production of glucose.

The present study differed from the studies cited above (5, 13, 14) with respect to both study population and study design. None of the other studies evaluated the suppression of GPR when the infant was consecutively exposed to both a low and a high infusion rate of glucose.

It is not possible to assess the enterohepatic contribution of glucose in this or in previous reports (5, 13, 14) or the duration of fast required to make such a contribution negligible. The infants of the present study were not given full amounts of oral feeding and in addition obtained the last meal several hours before the first sampling period. It thus appears unlikely that the oral intake should have more than a marginal effect on GPR in comparison with the marked changes performed with regard to rate of glucose infusion.

In contrast to the situation in newborns, glucose infusion has been found to cause a complete suppression of glucose production in both adult humans and adult animals (5–8). It has been discussed whether the defective glucose regulation in newborn infants and animals is due to lack of a hepatic response to an increased plasma glucose concentration, low insulin secretion, or hepatic or peripheral insulin resistance. Clamp experiments have shown incomplete suppression of glucose production in puppies despite very high insulin levels, whereas total suppression was found in adult dogs at corresponding insulin levels (6, 7). These results indicate the presence of insulin resistance in the puppies (6, 7). Corresponding observations were made by Cowett *et al.* (8) in comparisons of lambs and ewes.

The insulin levels found in the present investigation were low but were within the range reported in earlier studies (4, 5, 10, 13, 25). This is in contrast to what would be expected under conditions of insulin resistance. The low insulin response found even in the infants with a rather high degree of suppression of glucose production indicates the presence of additional regulatory factors. A decrease in stress hormone–induced glycogenolysis and gluconeogenesis after the high-rate glucose infusion could represent one such mechanism. On the other hand, the low degree of suppression found in some of the infants could be explained by a persistent influence of stress hormone on hepatic glucose output (3, 4, 11). It was not possible to quantitate stress hormones due to the small amounts of plasma available in our immature infants. Because all the infants were studied at least 1 d after birth, a persistent influence of stress hormones from delivery appears not to be a likely explanation. The infants were carefully selected according to their clinical condition. Thus, they were stable with regard to circulatory and respiratory parameters and had no concurrent illness. No drugs except for aminophylline had been given (seven of 10 infants). None of the infants showed any clinical or behavioral signs of stress. Administration of aminophylline may increase glycogenolysis, thereby causing a persistence of glucose production. In the present study, there were no differences between infants receiving aminophylline and those who were not, either with regard to production rates of glucose or to the degree of suppression of GPR. In a recent study on glucose production in preterm infants treated with theophylline, Fjeld *et. al.* (26) showed that there was no increase in GPR during treatment with theophylline.

The high-rate glucose infusion in the present study was chosen to correspond to the mean GPR found in previous studies of newborn infants (12, 16–18). The amount of glucose given was close to that administered routinely during the second day of life. In some of the infants, GPR might have exceeded the amount of glucose given. Consequently, a partly persistent glucose production should be necessary to meet the metabolic demands.

Under conditions of high turnover rates of glucose, the use of D-6,6-$^2H_2$-glucose might underestimate the GPR (27, 28). In the present study, the turnover rates were high during the high-rate infusion of glucose, and some degree of underestimation of GPR cannot be ruled out. If so, the degree of suppression may have been lower and even more incomplete than the results show.

Glucagon has its major role in glucose regulation in the presence of hypoglycemia. Because none of the studied infants were hypoglycemic, the lack of relationship between glucagon concentration and GPR or plasma glucose concentration is not surprising.

In conclusion, the results of the present study show that very immature infants respond to glucose infusion with incomplete and varying suppression of glucose production. Insulin seems to be more important than plasma glucose in regulating the glucose homeostasis, but addi-

tional factors such as stress hormones may also have an influence.

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