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Gluconeogenesis is Not Regulated by Either Glucose or Insulin in Extremely Low Birth Weight (ELBW) infants receiving Total Parenteral Nutrition

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Abstract

Objective—To determine potential factors regulating gluconeogenesis in Extremely Low Birth Weight (ELBW) infants receiving total parenteral nutrition (TPN).

Study design—Seven infants (0.824±0.068 kg; 25.4±0.5 wks; 3.3±0.2 d) were studied for 11 hours, with parenteral lipid and amino acids continued at pre-study rates. Glucose was supplied at pre-study rates for the first 5 h (period 1), was then reduced to 6 mg/kg·min for 1 h and further to ~3mg/kg·min for 5 h (period 2); 2.5 mg/kg·min of the glucose was replaced by [U-¹³C]glucose throughout the study for measurements of glucose production and gluconeogenesis. Concentrations of glucose, insulin, glucagon and cortisol were determined. Data obtained during periods 1 and 2 were compared using paired t-test.

Results—Gluconeogenesis and glucose production remained unchanged (2.12±0.23 vs. 1.84±0.25 mg/kg·min (NS) and 2.44±0.27 vs. 2.51±0.31 mg/kg·min (NS), respectively), despite a 60% reduction of the glucose infusion rate and subsequent 30% (124.7±10.8 to 82.6±8.9 mg/dL (p=0.009) and 70% (26.9±4.7 to 6.6±0.4 μU/mL (p=0.002)) decreases in glucose and insulin concentrations, respectively. Cortisol and glucagon concentrations remained unchanged.

Conclusion—In ELBW infants receiving TPN, gluconeogenesis is a continuous process that is not affected by infusion rates of glucose or concentrations of glucose or insulin.

The fine regulation of glucose metabolism in adults is primarily exerted by insulin and secondarily by its counter regulatory hormones glucagon, epinephrine, cortisol and growth hormone (1). In contrast, infants born prematurely are at high risk of disturbed glucose homeostasis resulting from limited substrate availability and potentially immature regulation of glucose metabolism. To prevent hypoglycemia and promote growth and development,

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infants born prematurely routinely receive total parenteral nutrition with a glucose supply exceeding the normal glucose turnover rates for infants [2-8] from their first days of life. However, their tolerance for parenteral glucose is low resulting in a frequent occurrence of hyperglycemia [2-3,9]. There is a paucity of data on which substrates and hormones regulate glucose production from its two sources, gluconeogenesis and glycogenolysis, during total parenteral nutrition (TPN) in preterm infants. This information is crucial to optimize nutritional strategies in this population.

We and others have demonstrated that preterm infants initiate gluconeogenesis within the first days of life both from endogenous body fuel stores and exogenous lipid and amino acid substrate [10-13]. Further, a variable suppression of glucose production was demonstrated in preterm infants receiving parenteral glucose [4,14-16]. In these studies, the infants received only glucose (i.e. no lipid and amino acids) and the partition of glucose production into its components gluconeogenesis and glycogenolysis were not measured.

Cowett et. al addressed the impact of insulin and its counter-regulatory hormones on glucose production in a systematic fashion in newborn lambs [17]. They conclude that insulin has a greater effect on glucose uptake than on glucose production and, glucagon, cortisol and growth hormone have no major effects on glucose production even during hyperinsulinemic hypoglycemia.

In a recent study, we demonstrated that in very low birth weight infants receiving routine TPN, glucose production was not completely suppressed and that gluconeogenesis constituted the major part of residual glucose production [18]. Building on these results, the present study was conducted during routine TPN providing glucose at high rates and in response to reducing the glucose infusion (as part of TPN) to half normal newborn glucose turnover rate. This approach enabled us to investigate potential factors regulating gluconeogenesis in extremely low birth weight (ELBW) infants. We hypothesized that gluconeogenesis would remain unchanged in response to reduction in the glucose supply and subsequent decreases in concentrations of glucose and insulin in ELBW infants receiving TPN due to imprecise hormonal regulation of glucose metabolism. In addition because normoglycemia had to be maintained in all infants, glucagon and cortisol, which have their effects under hypoglycemic conditions [19-21], were not expected to change. Therefore, these hormones would not affect gluconeogenesis during administration of TPN providing gluconeogenic substrates.

Methods

The protocol was reviewed and approved by the Institutional Review Board for Human Research at Baylor College of Medicine and the Advisory Board of the General Clinical Research Center at Texas Children's Hospital, Houston TX. The infants were recruited from the Neonatal Intensive Care Unit at Texas Children's Hospital and they were enrolled with written parental consent.

Seven premature infants (5 boys and 2 girls) fulfilling our inclusion criteria i.e. gestational age ≤ 29 wks; AGA (appropriate for gestational age); and absence of syndromes, anomalies and sepsis were studied. The mothers did not have diabetes or substance abuse. All mothers except one had received antenatal steroids. Four infants were delivered vaginally and three by cesarean section. The average Apgar score at 5 min was 7 with no infant having less than 5. Subject characteristics are shown in Table I. Further, the infants must be clinically stable on IPPV (Intermittent Positive Pressure Ventilation) or CPAP (Continuous Positive Airway Pressure) and not have a mother with diabetes or substance abuse.

At the time of the study, 6/7 infants were receiving caffeine and 2/7 dopamine (7.5 $\mu\text{g}/\text{kg}\cdot\text{min}$ and 10.0 $\mu\text{g}/\text{kg}\cdot\text{min}$, respectively). The infants had received antibiotics (Ampicillin + Gentamicin) prior to study, but in all of them antibiotics had been discontinued before start of the study. No infant had positive cultures or clinical signs of sepsis. In addition, concentrations of C-reactive protein (CRP) were measured to confirm the absence of severe illness. None of the infants had received insulin. The infants were clinically stable with oxygen saturation between 85 and 95% either on Intermittent Positive Pressure Ventilation (IPPV) (n=2) (20/5 cm H₂O and FiO₂ 0.28 ± 0.03) or Continuous Positive Airway Pressure (CPAP) (n=5) (8 ± 1 cm H₂O and FiO₂ 0.27 ± 0.04).

Parenteral nutrition

Amino acids (TrophAmine, Braun Medical Inc. Bethlehem, PA) were administered at the pre-study rates as ordered by the attending physician throughout the 11 hour study (2.04 ± 0.10 mg/kg·min; range 1.67-2.36 mg/kg·min corresponding to 2.4-3.4 g/kg·d).

Lipid (20% Intralipid, Kabivitrum, Stockholm, Sweden) was also administered at the pre-study rates throughout the 11 h study (1.12 ± 0.21 mg/kg·min; range 0.50-2.08 mg/kg·min = 0.72-3.00 g/kg·d).

To test the effect of a change in the glucose infusion rate on gluconeogenesis, we used two different glucose infusion rates. During the first 5 h of the study, glucose was given at the pre-study rates (8.4 ± 0.5 mg/kg·min; range 6.9-10.0 mg/kg·min corresponding to 10.0-14.4 g/kg·d) (denoted period 1). At study hour 5 (directly after a 5 hour blood sample), the glucose infusion rate was reduced step wise (to minimize counter regulatory responses) first to 6 mg/kg·min (i.e. corresponding to the normal glucose turn over rate of newborn infants [2-8]) for one hour and then further to 3.4 ± 0.2 mg/kg·min (range 2.9-4.1 mg/kg·min) for the remaining 5 h of the 11 h study period (denoted period 2).

Compounds labeled with stable isotopes

During the entire 11 hour study period, 2.5 ± 0.1 mg/kg·min of the above glucose infusion rates was replaced by [U-¹³C]glucose (metabolically equivalent to unlabelled natural glucose and used to measure glucose appearance rate and glucose production from gluconeogenesis and glycogenolysis). In addition, throughout the entire 11 hour study, [1-¹³C]leucine was given at a constant rate of 0.01 ± 0.0 mg/kg·min (0.6 ± 0.0 mg/kg·hr) to measure leucine turn over. The [U-¹³C]glucose (99 atom % ¹³C) and [1-¹³C]leucine (99 atom % ¹³C) were purchased sterile and pyrogen free from Cambridge Isotope Laboratories (Andover, MA) and were mixed and dissolved in normal saline at 12.5 and 0.5 mg/ml, respectively, by the Investigation Pharmacy at Texas Children's Hospital, Houston, TX. The tracer solutions were again tested for sterility and pyrogenicity and placed in sealed vials under sterile conditions by the investigational pharmacy. The TPN solution and the compounds labeled with stable isotopes were infused via umbilical venous catheters.

Blood sampling

Blood samples (a total of 3 ml/kg) were obtained at start of the study and at study hours 4.5, 5 (representing period 1), and 10.5 and 11 (representing period 2). The blood samples were obtained via umbilical artery catheters already in place for clinical care purposes.

Analyses

The isotopic enrichments and the mass isotopomer distribution of glucose and lactate during the [U-¹³C]glucose infusion were determined by gas chromatography – mass spectrometry (GCMS) (6890/5973 Agilent Technologies, Wilmington, DE). The pentaacetate and acetyl-pentafluorobenzyl derivative of glucose and lactate, respectively, were prepared as

previously described [10]. The blood samples were analyzed for blood glucose concentration using a glucose analyzer (YSI 2300 Stat Plus, YSI Inc. Yellow Springs, OH, USA). The ^{13}C isotopic enrichments of α -ketoisocaproic acid (KIC), the intracellular transamination product of leucine, was measured by GCMS using the oxime-terbutyldimethylsilyl derivative [10]

Insulin and Glucagon concentrations were determined by radioimmunoassays (Millipore, Billerica, MA). Other measurements were Cortisol, Adiponectin and C-reactive protein concentrations with non radioactive human ELISA kits; Cortisol kit from IBL Transatlantic, Toronto, ON and, Adiponectin and CRP from Millipore Corporation, Billerica, MA.

All kinetic measurements were performed under steady state conditions. Rates of total glucose appearance in plasma, glucose production and gluconeogenesis were measured at study hours 4.5 and 5 (period 1) and 10.5 and 11 (period 2). Total plasma glucose appearance rate (glucose Ra) was calculated from the M+6 enrichment of $[\text{U-}^{13}\text{C}]$ glucose in plasma using established isotope dilution equations [6,10].

Rate of glucose production (mg/kg·min) (GPR) = glucose Ra – exogenous glucose (labeled and unlabeled).

Fractional gluconeogenesis (i.e. gluconeogenesis as a fraction of glucose Ra) was calculated using $[\text{U-}^{13}\text{C}]$ glucose mass isotopomers distribution analyses (MIDA) [10-12,22]. Rates of gluconeogenesis were calculated as the product of total glucose appearance rate (glucose Ra) [10] and fractional gluconeogenesis

Rate of Gluconeogenesis (mg/kg·min) (GNG rate) = gluc Ra $\times$ GNG % Ra

Glycogenolysis was calculated by subtracting the rate of gluconeogenesis from the glucose production rate.

Rate of glycogenolysis (mg/kg·min) = GPR - GNG rate

Total leucine rate of appearance was calculated from the $[\text{U-}^{13}\text{C}]$ KIC enrichment using the “reciprocal pool” model [23]. Rate of appearance of endogenous leucine was calculated by subtracting the rate of infusion of exogenous leucine (TPN) from total leucine Ra and was considered an indicator of proteolysis. Leucine turnover values were converted to protein turnover assuming the content of leucine in body proteins is ~8% [24].

Statistical analyses—Absolute rates of gluconeogenesis, concentration of glucose, insulin, adiponectin, cortisol and glucagon obtained during the first 5 h period (period 1 representing high glucose infusion rate) were compared with those obtained during the last 5 h period (period 2 representing low glucose infusion rate) using paired t-tests. To account for multiple testing a p value <0.01 was used to define significance. Linear regression analysis was used to analyze relationships between measured variables i.e. gluconeogenesis, glucose appearance rate, glucose production rate, glucose concentration, and the various hormone concentrations. All results are provided as mean $\pm$ SE.

Results

Concentrations of glucose and insulin are depicted in Figure 1, and glucagon, cortisol, adiponectin and CRP in Table II. Glucose Appearance Rate averaged 10.88 ± 0.61 mg/kg·min (60.39 ± 3.39 $\mu\text{mol/kg·min}$) during period 1 and 5.92 ± 0.24 mg/kg·min (32.86 ± 1.33 $\mu\text{mol/kg·min}$) during period 2 ($p < 0.001$). Glucose Production Rates did not differ significantly between periods 1 and 2, 2.4 ± 0.3 mg/kg·min (13.5 ± 1.5 $\mu\text{mol/kg·min}$) vs. 2.5

± 0.3 mg/kg·min (13.9 ± 1.7 μ mol/kg·min) (NS). This shows that the difference in glucose appearance rate between periods 1 and 2 was solely a result of the difference in the glucose infusion rates. Rates of Gluconeogenesis and Glycogenolysis are shown in Figure 2 demonstrating that there was no difference in the rates between the two infusion rate periods (NS). Gluconeogenesis accounted for $89 \pm 5\%$ and $75 \pm 5\%$ of glucose production during periods 1 and 2, respectively (NS).

Total leucine rate of appearance was 0.73 ± 0.04 mg/kg·min (5.59 ± 0.33 μ mol/kg·min) during period 1 and 0.71 ± 0.03 mg/kg·min (5.38 ± 0.22 μ mol/kg·min) for period 2 (NS). Thus, the total rate of proteolysis was 13.2 ± 0.8 g/kg·day during period 1 and 12.7 ± 1.4 g/kg·day during period 2 (NS). Endogenous leucine Ra from proteolysis averaged 0.45 ± 0.04 mg/kg·min (3.40 ± 0.27 μ mol/kg·min) during period 1 and 0.42 ± 0.02 mg/kg·min (3.20 ± 0.16 μ mol/kg·min) for period 2, which corresponds to a total rate of endogenous proteolysis of 8.04 ± 0.65 g/kg·day during period 1 and 7.55 ± 0.37 g/kg·day during period 2 (NS).

Linear Regression Analyses did not show any effect of glucose infusion rate or glucose, insulin, glucagon, cortisol, adiponectin, and CRP concentrations on gluconeogenesis during either of the glucose infusion rate periods. During period 1, linear regression analysis demonstrated that glucose infusion rate explained 54% of the variation in glucose concentration, ($R^2=0.54$; $p=0.04$). Adding glucose production to the regression analysis, increased the R^2 value to 0.83 ($p=0.013$). During period 2, the glucose infusion rate was the same in all subjects by design (~ 3 mg/kg·min). Thus, as expected there was no relationship between glucose infusion rate and glucose concentration. However, blood glucose concentration was significantly related to the glucose production rate, $R^2=0.60$; $p=0.026$. The plasma glucose concentration was also not affected by glucagon, cortisol, adiponectin or CRP concentrations during either period 1 or 2.

Leucine rate of appearance from proteolysis and thus, total protein turnover was not affected by glucose infusion rate, plasma glucose, insulin or cortisol concentrations during the two glucose infusion rate periods.

Discussion

We recently reported that gluconeogenesis continues in preterm infants receiving routine TPN providing glucose at rates exceeding normal infant glucose turnover rates [18]. However, there are no reports on the regulation of gluconeogenesis in preterm infants. Our primary purpose was to determine whether insulin or glucose concentration regulates gluconeogenesis in ELBW infants receiving TPN. We demonstrated that gluconeogenesis remained unchanged and accounted for the major contribution to glucose production whether the infants received TPN with a glucose supply exceeding normal infant glucose turnover rate or the glucose infusion rate was reduced by 60%. In response to the reduction of the glucose infusion rate, glucose and insulin concentrations decreased by 30% and 70%, respectively (Figure 1). This clearly demonstrates that gluconeogenesis is not acutely affected by either insulin or glucose concentrations in ELBW infants receiving TPN. There was a strong relationship between the decreases in glucose and insulin concentrations between periods 1 and 2 showing that these immature infants were able to adjust insulin in response to the lower glucose concentrations.

The secondary objective was to investigate potential effects of the insulin counter regulatory hormones, glucagon and cortisol (limitations in the blood volumes that can be safely withdrawn in ELBW infants precluded measurement of epinephrine and growth hormone) on gluconeogenesis. We did not observe any changes in these hormones in response to the reduction in the glucose infusion rate and subsequent decreases in glucose and insulin

concentrations. Glucagon increased plasma free fatty acid and ketone body concentrations in humans even under conditions of elevated plasma insulin concentrations and stimulated synthesis of phosphoenolpyruvate, a key step of the gluconeogenic pathway [20,25]. Cortisol is known to be a counter regulatory hormone during prolonged hypoglycemia and stimulates gluconeogenesis by increasing the delivery of gluconeogenic substrates via lipolysis and proteolysis, and by enhancing the activity of key gluconeogenic enzymes [26-31]. Because our infants received lipid and amino acid substrate and remained normoglycemic, the lack of changes in glucagon and cortisol concentrations is not surprising. Under these conditions, the importance of these hormones in the regulation of gluconeogenesis is limited. In neonatal lambs, there was an imprecise response in the concentrations of glucagon and cortisol to hyperinsulinemic hypoglycemia and these hormones did not affect glucose production [17].

Incomplete suppression of glucose production in preterm infants was reported previously [4,14-15]. However, in these earlier studies the infants received only glucose i.e. no lipid and amino acids. This is an important difference because TPN is given soon after birth according to current nutritional guidelines. Further, the contribution from gluconeogenesis and glycogenolysis were not measured. Kalhan et al [13] did not observe any differences in gluconeogenesis between preterm infants receiving glucose alone or glucose plus lipid and amino acids using a method that included only the gluconeogenic contribution from pyruvate (i.e. glycerol is not included). In contrast, the method used in the present study provides an estimate of total gluconeogenesis i.e. new glucose generated from all non-carbohydrate sources including glycerol, which accounts for the major part of gluconeogenesis in preterm infants receiving TPN [10-11]. Thus, as expected, estimates of gluconeogenesis were lower in their study [13].

Gluconeogenesis remained unchanged in the infants despite the substantial reduction in glucose infusion rate. However, the infants were able to maintain normoglycemia by producing glucose primarily via the gluconeogenic pathway in addition to the low glucose supply confirming our previous reports [10-12]. This indicates that the rates of gluconeogenesis were already appropriately high during the low glucose infusion period but was not suppressed during the high glucose infusion rate period, despite exogenous glucose was supplied at rates exceeding the estimated needs of the infants.

Studies in human adults have shown fine regulation of glucose metabolism in response to intravenous glucose administration with complete or nearly complete suppression of glucose production [14,32-33]. Similarly, Cowett et. al reported that in adult sheep, glucose production was suppressed at relatively low glucose infusion rates (~6 mg/kg-min), while it was sustained in newborn lambs until the glucose infusion reached very high rates (~22 mg/kg-min) (34). In addition, there is evidence for the continuation of gluconeogenesis from lactate during a wide range of glucose and insulin infusion rates in newborn lambs [35]. Further, Farrag et. al showed that suppression of glucose production in preterm infants (32-33 wks) was only minimally affected by the insulin concentration during infusion of insulin at various rates [36]. In contrast, they observed a near complete suppression of glucose production in adults (~89%) during insulin infusion [36]. In the present study, gluconeogenesis accounted for ~80% of glucose production regardless of glucose infusion rate and resulting glucose concentration, suggesting persistent glucose production in extremely low birth weight infants is primarily a result of ongoing gluconeogenesis.

Hepatic insulin resistance could be a potential reason for the lack of suppression of gluconeogenesis in extremely low birth weight infants. However, insulin sensitivity is difficult to measure in these infants. Some studies have demonstrated correlation between adiponectin and insulin sensitivity [37-38]. Therefore, we measured adiponectin as a

potential indicator of insulin sensitivity. We found that adiponectin concentrations were similar during the two glucose infusion periods and were not related to rates of gluconeogenesis. The adiponectin concentrations found in our infants were of the same magnitude as those of the lean insulin sensitive adolescents we have previously reported [39]. This might indicate that sustained gluconeogenesis in these infants is not due to hepatic insulin resistance.

Our data also demonstrate that despite substantially higher insulin concentrations during infusion of glucose at high rates, blood glucose concentrations were elevated. We speculate that further increase in insulin would not have any major effects on the blood glucose concentration, which would support the reports of minimal clinical benefits and negative outcome of early insulin therapy in extremely low birth weight infants [40-41]. Additionally, similar protein turnover data during the two periods demonstrates that reduced supply of glucose as a part of TPN does not increase proteolysis in extremely low birth weight infants. This might imply that ongoing gluconeogenesis during TPN providing gluconeogenic substrate prevented a potential need of increased proteolysis to sustain gluconeogenesis to meet glucose demands when the glucose supply is low. In the absence of TPN, Hertz et al [16] showed that glucose supplied at or above normal infant glucose turnover rates did not affect proteolysis in extremely premature infants.

Because gluconeogenesis is an ongoing process enabling preterm infants receiving TPN to remain normoglycemic even during a glucose supply corresponding to half normal turnover rate, the glucose infusion rate is the primary factor that can be optimized to reduce the risk of hyperglycemia. Maintaining a glucose infusion rate corresponding to normal infant glucose turnover rates as part of total parenteral nutrition might be a feasible approach to prevent both hypo- and hyperglycemia and yet provide sufficient energy for growth in ELBW infants during their first days of life.

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Figure. 1.
Rates of glucose infusion and concentrations of insulin and glucose during the high and low glucose infusion rate periods.

Figure. 2. Rates of glucose production, gluconeogenesis and glycogenolysis during the high and low glucose infusion rate periods. Error bars refer to gluconeogenesis and glycogenolysis, respectively.

Table 1

Subject Characteristics

Subject #	Sex	Gestational Age wks	Birth Weight kg	Postnatal Age days
1	m	27	0.930	3
2	m	27	1.085	3
3	m	25	0.962	3
4	m	24	0.572	4
5	m	26	0.808	4
6	f	25	0.747	3
7	f	24	0.605	3
Mean $\pm$ SE		25.4 $\pm$ 0.5	0.824 $\pm$ 0.068	3.3 $\pm$ 0.2

Table 2

Concentrations of Glucagon, Cortisol, Adiponectin and CRP during high and low glucose infusion

	Period 1 High glucose infusion period	Period 2 Low glucose infusion period	p Value
Glucagon (pg/mL)	85.8 $\pm$ 8.2	94.8 $\pm$ 11.5	0.29
Cortisol (ng/mL)	151 $\pm$ 29	183 $\pm$ 39	0.09
Adiponectin (ng/mL)	9576 $\pm$ 1794	9686 $\pm$ 1383	0.92
CRP (μ g/mL)	1.26 $\pm$ 0.46	1.37 $\pm$ 0.55	0.45