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Original article

Reducing glucose infusion safely prevents hyperglycemia in post-surgical children☆

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SUMMARY

Background & aims: To investigate the effects of two different glucose infusions on glucose homeostasis and amino acid metabolism in post-surgical children.
Methods: This randomized crossover study evaluated glucose and amino acid metabolism in eight children (age 9.8 ± 1.9 months, weight 9.5 ± 1.1 kg) admitted to a pediatric intensive care unit in a tertiary university hospital after surgical correction for non-syndromal craniosynostosis. Patients were randomized to receive low (LG; 2.5 mg kg^{-1} min^{-1}) and standard (SG; 5.0 mg kg^{-1} min^{-1}) glucose infusion in a crossover setting. After a bolus (4 g kg^{-1}) of deuterium oxide, we conducted a primed, constant, 8 h tracer infusion with [6,6-$^2H_2$]Glucose, [1-^{13}C]Leucine, [ring-$^2H_5$]Phenylalanine and [3,3-$^2H_2$]Tyrosine.
Results: SG resulted in hyperglycemia (defined as > 6.1 mmol L^{-1}), while during LG plasma glucose levels were normoglycemic (5.9 ± 0.6 vs. 7.5 ± 1.7 mmol L^{-1}; LG vs. SG respectively, $p = 0.02$). Hypoglycemia did not occur during LG infusion. Endogenous glucose production was not fully suppressed during the hyperglycemic state under SG and increased with reduced glucose infusion (2.6 ± 1.5 vs. 1.1 ± 1.4 mg kg^{-1} min^{-1}; LG vs. SG; $p = 0.05$). Whole body protein balance derived from leucine and phenylalanine kinetics was slightly negative but not further affected with a decrease in glucose infusion.
Conclusions: The current recommended glucose infusion induces hyperglycemia in post-surgical children. A reduced glucose infusion safely reduced high glucose levels, while children were capable to sustain normoglycemia with increased endogenous glucose production. The reduced glucose infusion did not exacerbate the mild catabolic state in which the patients were.

1. Introduction

Plasma glucose levels are the resultant of exogenous glucose supply and endogenous glucose production on the one hand and glucose oxidation or storage as glycogen and triglycerides on the other. Critically ill children are at increased risk to a disturbance in this balance leading to hyper- as well as hypoglycemia.[1]

Hyperglycemia is a frequent complication and associated with increased morbidity and mortality in pediatric intensive care units (PICU's).[2] Notwithstanding the widespread implementation of tight glucose regimens,[3] concerns regarding hypoglycemia have been raised.[4] Recently, insulin therapy to achieve normoglycemia has been shown to improve morbidity as well as mortality in critically ill children, but also led to hypoglycemia (≤40 mg dL^{-1}–≤2.2 mmol L^{-1}) and severe hypoglycemia (≤31 mg dL^{-1}–≤1.7 mmol L^{-1}) in 87 (25%) and 17 (5%) children, respectively.[5]

The child's developing brain is more susceptible to hypoglycemia which can result in permanent damage.[6–8] Furthermore, young age is a risk factor for developing hypoglycemia, especially

Abbreviations: LG, low glucose infusion; SG, standard glucose infusion; PICU, pediatric intensive care unit; EGP, endogenous glucose production; APE, atom percent excess; MPE, mass percent excess; α-KIC, α-ketoisocaproate; NOLD, non-oxidative leucine disposal; NHPD, non-hydroxylation phenylalanine disposal; Ra, rate of appearance; Rd, rate of disappearance; CRP, C-reactive protein.

☆ This trial was registered in the Dutch trial register (www.trialregister.nl) under number NTR2079.

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when the child is ill.[9,10] This has led to a debate questioning the risks of insulin therapy in the pediatric population.[11,12]

An alternative to insulin therapy is to reduce the amount of glucose infusion in post-surgical children admitted to the PICU. This approach, however, also has two potential detrimental side-effects; an increased risk for hypoglycemia and an amplification of an already increased protein catabolism. Currently no data exist on the impact of different glucose infusions on glucose kinetics and amino acid metabolism in post-surgical children admitted to the PICU.

We hypothesized that in post-surgical children, reduced glucose infusion will improve plasma glucose levels without affecting glucose production rates or amino acid metabolism. Therefore, our first objective was to determine the impact of standard or low glucose infusions on glucose homeostasis and kinetics. Our second objective was to determine whether a low glucose infusion would affect protein and amino acid catabolism.

2. Methods

2.1. Patient characteristics

The study protocol was approved by the Medical Ethical Committee of the Erasmus Medical Center, Rotterdam, the Netherlands. The studies were performed in children after surgical correction for non-syndromal craniosynostosis within 6 h after admission to the PICU of the Erasmus Medical Center - Sophia Children's Hospital. Written informed consent was obtained from the parents. All were assessed by the Pediatric Logistic Organ Dysfunction (PELOD) score,[13] Pediatric Index of Mortality (PIM2)[14,15] and the Pediatric Risk of Mortality III (PRISM III) score,[16] which are validated measures of the severity of multiple organ dysfunction in PICU's. Patients with metabolic diseases, diabetes mellitus, primary liver, or renal failure were excluded.

2.2. Study design

The experimental design, shown in Fig. 1, consisted of a cross-over design, with a 4 h period of intravenous low glucose infusion (LG; 2.5 mg kg^{-1} min^{-1}) versus a 4 h period of standard glucose infusion (SG; 5.0 mg kg^{-1} min^{-1}).[17] Patients were randomized for the order of glucose infusion through a computer generated envelop. Laboratory personnel, nursing staff and investigators were blinded until analyses were finished. Six hours after admission ($t = 0$) to the PICU, an intravenous deuterium oxide infusion (^2H_2O; 4 gr kg^{-1}) was administered in 1 h to prime the body water pool. Two hours later ($t = 120$), after obtaining baseline blood samples, the intravenous glucose infusion as per standard care (4.0–6.0 mg kg^{-1} min^{-1})[17] was stopped and the study glucose infusion (SG or LG) started. Simultaneously, patients received a primed, continuous, 8 h intravenous tracer infusion (see below). Four hours after start of the tracer infusion ($t = 360$) the glucose infusion was switched.

2.3. Tracer infusion studies

All isotope tracers (Cambridge Isotope Laboratories, Andover, MA, USA) were tested for sterility and pyrogenicity after they were compounded at the investigational pharmacy at Erasmus Medical Center, Rotterdam, the Netherlands.

At $t = 120$, the bicarbonate pool was primed with 2.1 μmol kg^{-1} $NaH^{13}CO_2$, followed by a 8-h primed, continuous tracer infusion of [6,6-$^2H_2$]glucose (40 μmol kg^{-1}; 48 μmol kg^{-1} h^{-1}), L-[1-^{13}C] leucine (8 μmol kg^{-1}; 8 μmol kg^{-1} h^{-1}), [ring-$^2H_5$]Phenylalanine (5.4 μmol kg^{-1}; 4.1 μmol kg^{-1} h^{-1}), [3,3-$^2H_2$]Tyrosine (3.6 μmol kg^{-1}; 3.0 μmol kg^{-1} h^{-1}). The [ring-$^2H_5$]Phenylalanine derived tyrosine pool was primed with [ring-$^2H_4$]Tyrosine (2.5 μmol kg^{-1}).

2.4. Measurements and sample analysis

Blood samples were obtained at standard frequent intervals (Fig. 1), centrifuged (2 min 6000 rpg) and frozen at −80 °C until samples were analyzed.

Isotopic enrichment of deuterium in plasma body water was determined by isotope ratio mass spectrometry (Delta + XP IRMS Thermo Fisher, Bremen, Germany). Enrichments of glucose labeled with ^2H were measured by gas chromatography-mass spectrometry (GC–MS) (GC 6890, MS 5973N; Agilent Technologies, Wilmington, DE) using the penta-acetate derivative as previously described.[18,19]

Leucine kinetics were calculated from plasma alpha-ketoisocaproate (α-KIC) enrichment, which were determined by GC–MS after derivatization to butyldimethyl-silylquinoxalinol derivatives.[20] Plasma isotopic enrichments of [ring-$^2H_5$]Phenylalanine, [ring-$^2H_4$]Tyrosine and [3,3-$^2H_2$]Tyrosine were determined by GC–MS after using the *N*-ethoxycarbonylethylester derivative according to a modified method of Husek.[21]

Carbon dioxide production (VCO_2), oxygen (VO_2) consumption and respiratory quotient (RQ), were obtained with a metabolic monitor in canopy mode (Deltatrac™ I MBM-200, Datex Division Instrumentarium Corp., Finland). To determine the enrichment of $^{13}CO_2$ in whole blood, 1.5 mL of perchloric acid 10% was added to 1.5 mL of whole blood in a vacutainer to release the CO_2. The released gas was transferred to a vacuum impermeable glass tube and $^{13}CO_2$ was determined with IRMS.[22,23]

Plasma samples for glucose, insulin, cortisol, triglycerides, and free fatty acids were determined by standard in-house protocols. Plasma glucose levels >6.1 mmol L^{-1}(>110 mg dL^{-1}) were considered hyperglycemic.[24]

2.5. Calculations

Whole body kinetics of protein were calculated by conventional isotope dilution equations using a stochastic model during steady state enrichment[25] and glucose kinetics were estimated using the Steele equation,[26] based upon the last 40 min of both study periods.

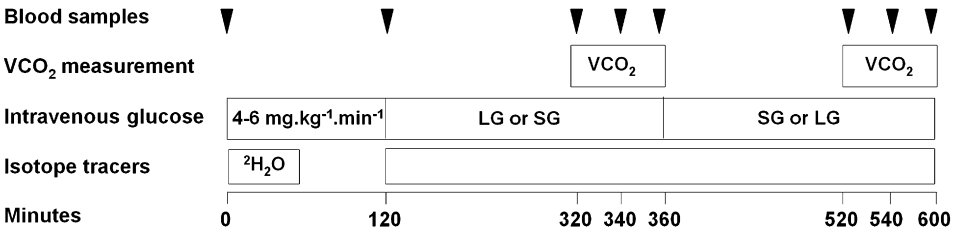

Fig. 1. Schematic presentation of the tracer infusion study in eight post-surgical infants receiving either low (LG, 2.5 mg kg^{-1} min^{-1}) or standard (SG, 5.0 mg kg^{-1} min^{-1}) glucose infusion. Black triangles indicate time points for plasma collection for laboratory parameters and isotopic enrichment measurements. Square boxes represent carbon dioxide production (VCO_2) measurements.

At steady state plateau, rate of appearance (Ra) equals the rate of disappearance (Rd) as follows:

$$\mathrm{Ra} = \mathrm{Rd} = i \times \left(\mathrm{E_{inf}}/\mathrm{E_{pl}} - 1\right) \quad (1)$$

where i is the infusion rate of the labeled tracer, $\mathrm{E_{inf}}$ is the tracer enrichment of the infusate and $\mathrm{E_{pl}}$ the tracer enrichment in plasma.

2.6. Glucose kinetics

Endogenous glucose production (EGP) rate is calculated as follows:

$$\mathrm{EGP} = \mathrm{Ra_{Glucose}} - \mathrm{GIR} \quad (2)$$

where GIR is the total glucose infusion rate in mg kg^{-1} min^{-1}.

Fractional gluconeogenesis is calculated as previously described.[19] Briefly, the average enrichment of ^2H on each glucose carbon was calculated with the following equation:

$$\mathrm{Average\ (M+1)d} = (\mathrm{M+1})\mathrm{d}_{(m/z169)}/6 \quad (3)$$

where $(\mathrm{M+1})\mathrm{d}_{(m/z169)}$ is the M + 1 enrichment of deuterium of glucose measured using m/z 170/169 and "6" is the number of ^2H labeling sites on the m/z 169 fragment of glucose.

Because body water is the precursor pool for deuterium or hydrogen, the extent of deuterium labeling of glucose during the gluconeogenic process when ^2H_2O is infused is a measure of fractional gluconeogenesis. Therefore, with the average deuterium enrichment in m/z 170/169 for calculating fractional gluconeogenesis (FracGNG), the equation is

$$\mathrm{FracGNG} = \mathrm{average\ (M+1)d}/\mathrm{E_{H_2O}} \quad (4)$$

where $\mathrm{E_{H2O}}$ is the deuterium enrichment in body water.

The absolute rate of appearance of plasma glucose from gluconeogenesis ($\mathrm{Ra_{GNG}}$) and glycogenolysis were calculated as follows:

$$\mathrm{Gluconeogenesis} = \mathrm{Ra_{Gluc}} \times \mathrm{FracGNG} \quad (5)$$

$$\mathrm{Glycogenolysis} = \mathrm{EGP} - \mathrm{Gluconeogenesis} \quad (6)$$

2.7. Amino acid metabolism

Whole body leucine, phenylalanine and tyrosine fluxes were calculated according to (*1*) as previously described.[27–29]

Leucine oxidation rates were calculated as follows;

$$\mathrm{LeucineOx} = \mathrm{VCO_2} \times \left(\mathrm{E^{13}CO_2}/69.18\right) \Big/ [^{13}\mathrm{C}]\alpha - \mathrm{KIC} \quad (7)$$

where 69.18 is the $^{13}CO_2$ refraction correction factor for critically ill children.[30] VCO_2 is measured in mL/min and converted to mMol/h by multiplying by 60 min and dividing by 22.4; the number of 1 in 1 mol of an ideal gas at standard temperature and pressure to convert to mL/min. Non-oxidative leucine disposal (NOLD; leucine converted into protein synthesis) was calculated as follows:

$$\mathrm{NOLD} = \mathrm{Ra_{leu}} - \mathrm{LeucineOx} \quad (8)$$

Phenylalanine hydroxylation (rate of phenylalanine conversion to tyrosine) was calculated as follows;[31,32]

$$\mathrm{Hydroxylation} = \mathrm{Ra_{tyr}} \times \left(\mathrm{E_{[2H4]tyr}}/\mathrm{E_{[2H5]Phe}}\right) \times \left(\mathrm{Ra_{phe}}/\mathrm{i_{phe}} + \mathrm{Ra_{phe}}\right) \times 2.2 \quad (9)$$

where $\mathrm{Ra_{phe}}$ and $\mathrm{Ra_{tyr}}$ are the phenylalanine and tyrosine fluxes; $\mathrm{E_{[2H4]tyr}}$ and $\mathrm{E_{[2H5]Phe}}$ are the plasma enrichments; and $\mathrm{i_{phe}}$ is the infusion rate of labeled phenylalanine and the term $(\mathrm{Ra_{phe}}/(\mathrm{i_{phe}} + \mathrm{Ra_{phe}})$ corrects for the contribution of the tracer infusion to $\mathrm{Ra_{phe}}$. The factor 2.2 is to correct for the secondary deuterium-isotope kinetic effect for in vivo hydroxylation in fasted state as described and validated previously.[31,32] Non-hydroxylation phenylalanine disposal (NHPD; phenylalanine converted into protein synthesis) was calculated as follows:

$$\mathrm{NHPD} = \mathrm{Ra_{phe}} - \mathrm{Hydroxylation} \quad (10)$$

2.8. Whole body protein metabolism

Under the assumption that 1 g of protein contains approximately 621 μmol of leucine[33] and 280 μmol of phenylalanine,[34] it is then possible to convert leucine and phenylalanine kinetics (μmol kg^{-1} h^{-1}) into protein kinetics (g kg^{-1} d^{-1}). Whole body protein turnover was calculated from the model described by Golden and Waterlow.[35]

2.9. Statistical analysis

A prospective power analysis revealed that 8 patients with complete data, would detect a difference of 20% of plasma glucose levels (80% power, type I error of 5%). The Shapiro–Wilk normality test was used to determine whether data were normally distributed. Comparison between the two different glucose infusions at both infusion rates were made using the paired student's t-test. For non-parametric data the Wilcoxon matched pairs test was used. Data are presented as the mean ± standard deviation unless non-parametric in which case they are presented as median and interquartile range. Statistical significance was considered at $p < 0.05$. Repeated measures ANOVA were used to analyze the effect of glucose infusion on parameters of glucose and protein metabolism over time and between LG and SG. Data were analyzed with Graphpad Prism 5.0.3 (Graphpad Software, La Jolla, CA., USA). This trial was registered in the Dutch trial register (www.trialregister.nl) under number NTR2079.

3. Results

3.1. Patient characteristics

Eight children (9.8 ± 1.9 months) admitted to the PICU after surgical correction for non-syndromal craniosynostosis were included (Table 1). All patients were hemodynamically stable without vasoactive drugs and breathing spontaneously with a $FiO_2 < 0.6$. They received opioids or acetaminophen as pain relief and were not sedated or receiving muscle relaxation. Patients did not receive (par)enteral nutrition other than intravenous glucose as per standard care (4–6 mg kg^{-1} min^{-1}).[17]

3.2. Laboratory values and hormone concentrations

Patients were hyperglycemic during standard glucose infusion (SG), while during LG plasma glucose levels were lower and normoglycemic (Fig. 2, Table 1). LG did not cause hypoglycemia, the lowest plasma glucose was 5.1 mmol L^{-1} (91.8 mg dL^{-1}). Insulin plasma concentrations did not differ significantly (Table 1).

3.3. Stable isotope kinetics

Steady state conditions were achieved during the last 40 min of each study period (data not shown).

Table 1
Demographic and nutritional data of 8 post-surgical children.[a]

Age (months)	9.8 ± 1.9		
Gender (male: female)	6:2		
Weight (kg.)	9.5 ± 1.1		
Height (cm)	74.3 ± 3.0		
PELOD[b]	10.1 ± 7.6		
PRISM III[c]	7.4 ± 3.7		
PIM2[d]	14.2 ± 2.8		
	LG[e]	SG[f]	*P* value
Glucose infusion ($mg\ kg^{-1}\ min^{-1}$)	2.6 ± 0.1	5.2 ± 0.1	<0.0001
VO_2 ($mL\ min^{-1}$)	69 ± 24	67 ± 17	0.79
VCO_2 ($mL\ min^{-1}$)	59 ± 11	55 ± 14	0.29
Resting energy expenditure ($kcal\ kg^{-1}\ d^{-1}$)	49.8 ± 17.6	49.7 ± 14.9	0.98
Respiratory quotient	0.88 ± 0.14	0.83 ± 0.10	0.37
Caloric intake ($kcal\ kg^{-1}\ day^{-1}$)	12.7 ± 0.2	25.3 ± 0.5	<0.0001
Caloric intake (%)[g]	24 ± 1	47 ± 2	<0.0001
Glucose ($mmol\ L^{-1}$)	5.9 ± 0.6	7.5 ± 1.7	0.02
Triglycerides ($mmol\ L^{-1}$)	0.38 ± 0.25	0.43 ± 0.20	0.54
Free fatty acids ($mmol\ L^{-1}$)	0.72 ± 0.20	0.63 ± 0.12	0.34
C-reactive protein ($mg\ dL^{-1}$)	24 ± 13	26 ± 16	0.61
Cortisol ($nmol\ L^{-1}$)	649 ± 160	681 ± 205	0.77
Insulin ($pmol\ L^{-1}$)	64 ± 48	90 ± 51	0.16

[a] All values are mean ± SD.
[b] PELOD = Pediatric Logistic Organ Dysfunction (17).
[c] PRISM III = Pediatric Risk of Mortality III (20).
[d] PIM2 = Pediatric index of Mortality (18, 19).
[e] LG = Low glucose.
[f] SG = Standard glucose.
[g] Caloric intake as percentage of requirements according to the Schofield equation (59).

3.3.1. Glucose kinetics

The deuterium enrichment of body water was 0.59 ± 0.02 and 0.58 ± 0.03 MPE, at LG and SG respectively. Glucose Ra did not differ between glucose protocols (Table 2). EGP increased during LG (Fig. 2, Table 2). Absolute gluconeogenesis and glycogenolysis were not significantly different ($p = 0.08$) and glycogenolysis was not significantly different from zero (Table 2).

3.3.2. Amino acid kinetics

Leucine Ra, oxidation and NOLD did not differ (Table 3). Phenylalanine and tyrosine Ra did not differ. Phenylalanine hydroxylation was significantly higher at the lower glucose infusion rate (Table 3). However, NHPD (Table 3) and the phenylalanine hydroxylation fraction of the total phenylalanine Rd did not differ (12 ± 3% vs. 11 ± 3%; LG vs. SG, $p = 0.07$).

3.3.3. Protein metabolism

Whole body protein metabolism corresponding with leucine kinetics was as follows (Fig. 3). Protein synthesis (4.7 ± 0.5 vs. 4.8 ± 0.8 $g\ kg^{-1}\ d^{-1}$) and breakdown (5.9 ± 0.6 vs. 5.8 ± 0.6 $g\ kg^{-1}\ d^{-1}$) did not differ between LG and SG respectively. Protein balance was negative and did not differ (−1.2 ± 0.8 vs. −1.0 ± 0.6 $g\ kg^{-1}\ d^{-1}$, LG vs. SG). Whole body protein metabolism corresponding with phenylalanine and tyrosine kinetics was as follows (Fig. 3). Protein synthesis (5.3 ± 0.6 vs. 5.2 ± 0.5 $g\ kg^{-1}\ d^{-1}$) and breakdown (5.6 ± 0.5 vs. 5.4 ± 0.4 $g\ kg^{-1}\ d^{-1}$) did not differ between LG and SG respectively. Protein balance was negative and showed a statistically significant but not clinical relevant difference (−0.3 ± 0.1 vs. −0.2 ± 0.1 $g\ kg^{-1}\ d^{-1}$; LG vs. SG, $p = 0.04$).

4. Discussion

Tight glucose control improves morbidity and mortality in critically ill children, although hypoglycemia is a frequent and serious side effect of insulin therapy.[5] In this study we showed that in post-surgical children, normoglycemia could be achieved by a reduced glucose infusion, without occurrence of hypoglycemia, although no conclusion can be drawn outside our study intervention period. The reduced glucose infusion, half of what is considered standard practice for age,[17] can be considered an alternative to insulin therapy in the initial phase of glycemic management. Additionally we observed that EGP was not fully suppressed, despite high plasma glucose levels. Moreover, reducing the glucose infusion induced an increase in EGP without a significant increase in either gluconeogenesis or glycogenolysis (both $p = 0.08$). Furthermore, reduced glucose did not notably affect the negative whole body protein balance, measured with leucine and phenylalanine kinetics.

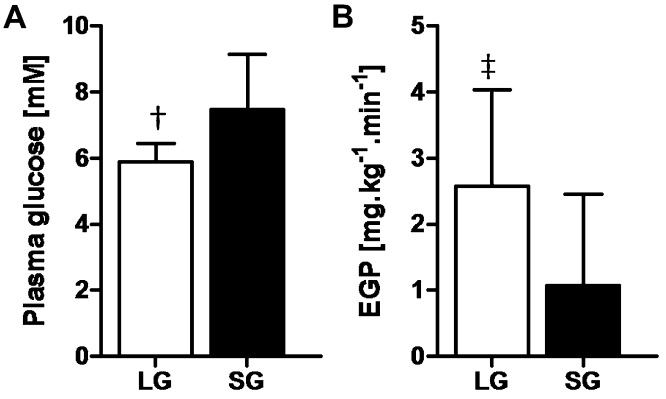

Fig. 2. Glucose metabolism during low and standard glucose infusion in eight post-surgical infants. Panel A. Plasma glucose concentration, mmol L^{-1}, mean ± SD, †$p = 0.02$, Panel B. Endogenous glucose production mg kg^{-1} min^{-1}, mean ± SD, ‡$p = 0.05$.

Our study is the first to show the feasibility of reduced glucose infusion in post-surgical children to prevent or treat hyperglycemia. We found that during LG plasma glucose levels were lower and normoglycemic, not due to an increased rate of disposal or decreased glucose production, but solely through a reduced glucose infusion rate. Due to the paucity of data in children it is difficult to define lower limits of glucose infusion. Current recommendations are based largely upon data for children extracted from the relation between glucose intake and 1) glucose uptake by the brain, 2) glucose oxidation, 3) endogenous glucose production (EGP), and 4) protein and amino acid catabolism.[17] Although conditions are different in post-surgical children, we used these considerations to determine whether LG was within safe limits in our population. We acknowledge that we did not quantify cerebral glucose uptake. Additionally, exact determination of glucose oxidation with [^{13}C]

Table 2
Glucose kinetics in eight post-surgical infants during two different glucose infusions.[a]

	LG[d]	SG[e]	*P* value
Glucose Ra[b]/Rd[c] $mg\ kg^{-1}\ min^{-1}$	5.3 ± 1.5	6.4 ± 1.5	0.14
Endogenous Glucose Production $mg\ kg^{-1}\ min^{-1}$	2.6 ± 1.5	1.1 ± 1.4	0.05
Fractional gluconeogenesis % of Ra[b]	43 ± 2	29 ± 7	<0.01
Absolute Gluconeogenesis $mg\ kg^{-1}\ min^{-1}$	2.3 ± 0.6	1.8 ± 0.4	0.08
Glycogenolysis $mg\ kg^{-1}\ min^{-1}$	0.3 ± 0.9	−0.7 ± 1.1	0.08

[a] All values are depicted as mean ± SD.
[b] Ra; rate of appearance.
[c] Rd; rate of disappearance.
[d] LG; Low glucose.
[e] SG; Standard glucose.

Table 3
Leucine, phenylalanine and tyrosine kinetics in eight post-surgical infants during two different glucose infusions.[a]

	LG[b]	SG[c]	*P* value
Leucine Ra[d]	160 ± 15	158 ± 16	0.68
Leucine oxidation	33 (22–82)	29 (22–62)	0.38
NOLD[e]	122 ± 14	124 ± 20	0.76
Phenylalanine Ra	70 ± 6	68 ± 5	0.25
Tyrosine Ra	39 ± 2	37 ± 2	0.06
Phenylalanine hydroxylation	8.4 ± 1.7	7.4 ± 1.6	0.04
NHPD[f]	61 ± 6	61 ± 6	1.0

[a] All values are measured in μmol kg^{-1} h^{-1} and depicted in mean ± SD, except for Leucine oxidation which is depicted in median (range).
[b] LG; Low glucose.
[c] SG; Standard glucose.
[d] Ra; rate of appearance.
[e] NOLD; non-oxidative leucine disposal.
[f] NHPD; non-hydroxylation phenylalanine disposal.

tracer data was not possible in our study, because of interference with our [^{13}C]Leucine tracer. Therefore, we cannot provide clear insight in the impact of LG on cerebral glucose uptake and utilization or oxidation. However, glucose oxidation calculated from the oxygen consumption and carbon dioxide production did not change. Of greater importance, hypoglycemia did not occur during low glucose infusion. This suggests that the reduced glucose intake did not negatively impact cerebral glucose uptake or utilization.

EGP rates in our infants, receiving 2.5 mg kg^{-1} min^{-1}, were lower than those measured in healthy infants, fasted for 8–9 h (7.1 ± 0.3 mg kg^{-1} min^{-1}).[36] So, although an increased EGP was observed during LG, their production was not at their full potential. During both glucose infusions glycogenolysis was almost completely blocked. Glycogenolysis was probably suppressed by elevated insulin levels and suppressed glucagon levels during glucose infusion,[37] although the latter could not be measured in our study due to blood draw limitations. Considering the exogenous glucose our patients received it is not likely that glycogen stores were already depleted. Therefore, it appears safe to conclude that our infants were capable of sustaining normoglycemia, without a direct risk of developing hypoglycemia. However, conclusions cannot be drawn for a longer period beyond the study period of reduced glucose infusion we presented.

Of interest, even during SG, despite supraphysiological glucose and insulin levels, EGP was not fully suppressed. During both glucose infusions, gluconeogenesis constituted almost all of the EGP and it appears that gluconeogenesis in contrast to glycogenolysis was unresponsive to insulin, high glucose concentrations and/or infusion. A clear association exists between increased contribution of gluconeogenesis and insulin resistance.[38] The expression of phosphoenolpyruvate-carboxy-kinase (PEPCK), the rate-limiting enzyme for gluconeogenesis, is increased and less sensitive to insulin during critical illness.[39] Therefore, we hypothesize that the increase in EGP and moreover gluconeogenesis during LG does not indicate that LG was below the threshold, but that it might indicate a certain state of hepatic insulin resistance in these post-surgical infants.

Our study was not able to show which precursors (amino acids, glycerol or lactate) were predominantly used to fuel gluconeogenesis. Although our static measurements of lipid metabolism (FFA and triglycerides) did not differ (Table 1), these results do not exclude a change in lipolysis, because we didn't confirm this by means of glycerol isotope tracer infusions. Another important gluconeogenic precursor is lactate. However, as our patients were hemodynamically stable with good organ perfusion, lactate levels are not expected to have increased during either intervention. Finally, protein or amino acid catabolism were not significantly increased.

Although LG did not affect leucine kinetics and more specifically oxidation, phenylalanine hydroxylation was slightly but statistically significant higher during LG. Increased availability of plasma amino acids, a known stimulant of hydroxylation,[40–42] can be ruled out as an explanation. Furthermore, a physiological response to cope with an energy shortage is unlikely as the leucine oxidation was unaffected. Possibly, phenylalanine catabolism was induced to provide gluconeogenic precursors. Products derived from phenylalanine hydroxylation can be used for gluconeogenesis, while oxidized leucine can merely be used for the ketogenic pathway.[43] This could explain the difference in catabolism between phenylalanine and leucine. These slight differences in amino acid or protein metabolism during low glucose infusion are not likely to have clinical relevance.

Whole body protein balance was as expected slightly negative during both study periods and consistent with those obtained from septic infants and children.[33,34] In contrast with our data, normoglycemia, independent of plasma insulin levels, improved protein turnover after abdominal surgery in adults.[44] Our different results may be explained by differences in patient populations and the approach used. Furthermore, there are tissue and age specific differences in the metabolic processes.[45] However, consistent with our data, it has been shown in neonates that different glucose

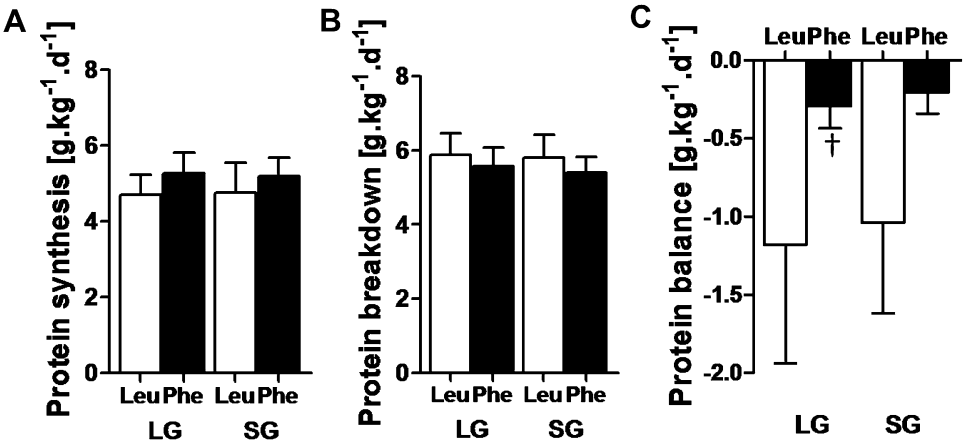

Fig. 3. Whole body protein metabolism during low and standard glucose infusion in eight post-surgical infants. Leu = leucine, Phe = Phenylalanine. Panel A. protein synthesis, Panel B. protein breakdown, Panel C. protein balance; Values are g kg^{-1} d^{-1}, mean ± SD, $^{\dagger}p = 0.04$; LG vs. SG.

infusions do not affect protein turnover.[46] During critical illness a deficiency in energy supply is not solely responsible for protein catabolism.[47] Proteolysis during critical illness is usually caused by activation of the ubiquitin-proteasome proteolytic pathway (UPP) in muscle initiated by activation of caspase 3,[48,49] and pathophysiological triggers include activation of lysosome-[50] and calpain-dependent pathways.[51] Furthermore, there appears to be a link between muscle wasting and insulin resistance.[52–54]

There are some limitations to our study, some of them inherent to studying critically ill children. Our sample size was small and conclusions from our study are restricted to post-surgical infants. Substrate metabolism greatly differs between infants, children and adolescents both for glucose and amino acid metabolism.[17,45] Furthermore, various diagnoses, such as trauma, burns or sepsis, as well as differences between single and multi-organ failure yield a different response. We also acknowledge that the negative glycogenolysis rates are physiological not possible. This might be explained by a consistent underestimation of the EGP by the tracer model, as part of the diluted tracer pool, due to newly produced glucose, is taken up by the liver again.[55] These limitations, however, do not weaken the conclusions drawn from our study. Finally, although our study does not provide data on reduced glucose infusion for longer than 4 h this approach appears to be safe in the initial postoperative phase which duration is individually dependent but usually lasts 6-24 h. Confirmatory studies with reduced glucose infusion during this study period are warranted.

5. Conclusion

Reduced glucose infusion, half of what is considered standard practice for age, in the initial post-surgical phase reduced high glucose levels in infants without occurrence of hypoglycemia. Additionally we observed that the endogenous glucose production was not fully suppressed and almost entirely relied on gluconeogenesis, despite high plasma glucose levels and exogenous glucose infusion. Furthermore, protein and amino acid catabolism was not exacerbated during the reduced glucose infusion. Although further studies on the optimal time period and (long term) clinical implications are warranted, reduced glucose infusion can be considered an alternative to insulin therapy in the initial glycemic postoperative management.

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Statement of authorship

S.V. Study concept and design; acquisition of data; analysis and interpretation of data; statistical analysis; drafting of the manuscript.

C.d.B. Acquisition of data; analysis and interpretation of data; critical revision of the manuscript.

H.S. Technical and material support; analysis and interpretation of data; critical revision of the manuscript.

S.C. Technical and material support; analysis and interpretation of data; critical revision of the manuscript.

J.V. Study concept and design.

J.v.G. Study concept and design; obtained funding; technical and material support; analysis and interpretation of data; critical revision of the manuscript; study supervision.

K.J. Study concept and design; obtained funding; analysis and interpretation of data; statistical analysis; critical revision of the manuscript; study supervision.

Conflict of Interest

All authors have not disclosed any potential conflicts of interest.

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