Gluconeogenesis in Very Low Birth Weight Infants Receiving Total Parenteral Nutrition

Agneta L. Sunehag, Morey W. Haymond, Richard J. Schanler, Peter J. Reeds, and Dennis M. Bier

Very low birth weight (VLBW) infants are dependent on total parenteral nutrition (TPN) to prevent hypoglycemia and provide a sufficient energy intake. However, diminished tolerance for parenteral glucose delivered at high rates frequently provokes hyperglycemia. We hypothesized that when their glucose supply is reduced to prevent hyperglycemia, VLBW infants can maintain normoglycemia via gluconeogenesis from glycerol and amino acids. Twenty infants born at 27 ± 0.2 (mean ± SE) gestational weeks and having a birth weight of 996 ± 28 g, received lipids (1.6 ± 0.1 mg · kg^{-1} · min^{-1}), protein (2.2 ± 0.1 mg · kg^{-1} · min^{-1}), and glucose (3.1 ± 0.1 mg · kg^{-1} · min^{-1} [17.1 ± 0.2 μmol · kg^{-1} · min^{-1}]) parenterally over a period of 8–12 h on day 5.0 ± 0.2 of life. Gluconeogenesis was estimated using [U-^{13}C]glucose (n = 8) or [2-^{13}C]glycerol (n = 6) and mass isotopomer distribution analysis (MIDA), or ^2H_2O (n = 6) and the rate of deuterium incorporation in carbon 6 of glucose. Blood glucose averaged 3.0 ± 0.1 mmol/l; plasma glucose appearance rate (glucose Ra), 28.8 ± 1.1 μmol · kg^{-1} · min^{-1}; and glucose production rate (GPR), 10.7 ± 1.0 μmol · kg^{-1} · min^{-1}. The [U-^{13}C]glucose and [2-^{13}C]glycerol tracers provided similar estimates of gluconeogenesis, averaging 28 ± 2 and 26 ± 2% of glucose *R*a and 72 ± 5 and 73 ± 9% of GPR, respectively. Glycerol contributed 64 ± 5% of total gluconeogenesis. Gluconeogenesis measured by ^2H_2O, which does not include the contribution from glycerol, was comparable to the nonglycerol fraction of gluconeogenesis derived by the [2-^{13}C]glycerol MIDA. We conclude that in VLBW infants receiving TPN, normoglycemia was maintained during reduced glucose infusion by glucose production primarily derived from gluconeogenesis, and that glycerol was the principal gluconeogenic substrate. ***Diabetes*** **48:791–800, 1999**

From the U.S. Department of Agriculture/Agricultural Research Service Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, Texas.

Address correspondence and reprint requests to Dr. Agneta L. Sunehag, Children's Nutrition Research Center, 1100 Bates St., Houston, TX 77030. E-mail: asunehag@bcm.tmc.edu.

Received for publication 2 June 1998 and accepted in revised form 8 December 1998.

CV, coefficient of variation; GCMS, gas chromatography–mass spectrometry; GPR, glucose production rate; HFBA, heptafluorobutyric anhydride; HMT, hexamethylenetetramine; KIC, α-ketoisocaproic acid; MIDA, mass isotopomer distribution analysis; *R*a, appearance rate; RIA, radioimmunoassay; TPN, total parenteral nutrition; VLBW, very low birth weight.

During the last decade, improvements in the management of premature infants have resulted in a dramatic decrease of the mortality rate in this population (1). Thus, 82–91% of infants weighing 750–1,250 g survive in the U.S. today (1), but knowledge about their metabolic needs and macronutrient requirements is limited at best.

Because glucose is the principal fuel for the brain, and erythrocytes and prolonged or repeated hypoglycemia may have severe neurodevelopmental consequences (2), appropriate delivery of carbohydrate fuels has important clinical implications, particularly in very low birth weight (VLBW) infants, who have high glucose requirements as well as high energy requirements because their brains are large in relation to their body weight (3,4). Despite the glucose and energy demands, infants born prematurely have diminished substrate stores (5,6), potentially rate-limiting activity of key gluconeogenic enzymes regulating gluconeogenesis (7), and intolerance to full enteral feeding. Therefore, these infants have a high risk for hypoglycemia. To prevent hypoglycemia, and to provide sufficient calories for energy expenditure and growth (8), premature infants are routinely given parenteral nutrition solutions, which deliver glucose at rates significantly higher than previously measured glucose turnover rates (4,9–12). The net advantages of this approach, however, are not entirely clear because VLBW infants have limited amounts of insulin-dependent tissues (13–15), glucose transporter systems that are not fully developed (assuming that the immature human infant is similar to the immature rat) (16,17), and insufficient insulin secretory responses to glucose provocation compared with older infants and adults (18–23). This combination of effects leads to a reduced tolerance for parenterally administered glucose, frequently resulting in hyperglycemia (21–27) with consequent adverse urinary loss of glucose, water, and electrolytes. High glucose loads may also increase energy expenditure and CO_2 production (28), a particular problem in infants whose pulmonary function may already be compromised.

We do not know the optimal composition of parenteral nutrition solutions that would provide VLBW infants with an adequate energy intake while maintaining normoglycemia. One approach to satisfying these conditions would be to use the infants' capacity for gluconeogenesis and substitute parenteral lipids and amino acids for part of the routinely given glucose.

We and others have previously demonstrated that term and preterm newborns can use endogenous stores of glycerol (29–33) and alanine (34) to produce glucose. These studies, however, estimated only the minimal contributions of glycerol and alanine, respectively, to glucose production, because the

methods used permitted evaluation of only one substrate at a time and did not provide correction for the dilution of infused isotope within the Krebs cycle and the gluconeogenic pathway. Three new approaches for estimating gluconeogenesis that have been described recently use [U-^{13}C]glucose or [2-^{13}C]glycerol and mass isotopomer distribution analysis (MIDA) of glucose, or ^2H_2O with assessment of deuterium enrichment in carbon 5 or 6 of glucose (35–42). Each of these new methods can potentially provide the information lacking in the previously described approaches, but each also has its own individual modeling assumptions. Therefore, there remains active debate about the relative validity of the methods for assessing gluconeogenic rates in vivo in humans (42–46).

To date, none of these methods has been used extensively, in either adults or children. In newborn infants, only the [U-^{13}C]glucose method has been previously employed in a single study (P.J.R. and colleagues [47]). However, this investigation is not directly comparable to the present one, because the infants were studied for only 4 h while receiving a considerable amount of glucose (5 mg · kg^{-1} · min^{-1}) but no exogenous amino acids. In adults, the three methods have been used individually (35,38–43), but there are no published reports in which all three methods have been compared in the same study. To address this issue, we have used all three methods to quantify gluconeogenesis in a population of VLBW infants who were receiving parenterally supplied amino acids and lipids but a limited exogenous glucose supply, to permit the study of gluconeogenesis while maintaining normoglycemia.

Thus, the aims of the present study were *1*) to compare three recently described methods using stable isotopes (35–42) to estimate gluconeogenesis in VLBW infants and *2*) to determine how VLBW infants use the gluconeogenic pathway to maintain normoglycemia while receiving total parenteral nutrition (TPN), with glucose delivered at a rate corresponding to half the normal glucose turnover rate over a period of 8–12 h.

We hypothesized that under these conditions, *1*) normoglycemia would be maintained by glucose production derived primarily from gluconeogenesis; *2*) glycerol would be a significant gluconeogenic substrate; *3*) the [U-^{13}C]glucose and [2-^{13}C]glycerol methods would provide similar estimates of gluconeogenesis, and ^2H_2O and incorporation of deuterium in carbon 6 of glucose would underestimate total gluconeogenesis in proportion to the amount of glucose contributed by glycerol; and *4*) whole-body protein breakdown would not be increased.

RESEARCH DESIGN AND METHODS

Subjects. The protocol was approved by the Institutional Review Board for Human Subject Research at Baylor College of Medicine in Houston, Texas. The subjects, recruited from the neonatal intensive care unit at Texas Children's Hospital, were enrolled after written consent was obtained from at least one parent.

The subject characteristics are shown in Table 1. We studied 20 infants (9 boys and 11 girls) without congenital anomalies or discernible diseases. Of these infants, eight were black, seven were Caucasian, and five were Hispanic. None had a diabetic mother. All infants were born at or before a gestational age of 29 weeks, weighed between 726 and 1,180 g at birth, and were appropriate for gestational age (48,49). Eleven of the mothers had received antenatal steroids (betamethasone, one to five doses of 12 mg each) (Table 1). None of the infants experienced acidosis or hypoxia at birth, and Apgar scores at 5 min (determined by neonatologists not involved in the study) were >5 in all infants (Table 1). All infants had received two to four doses (4 ml/kg) of surfactant (Survanta; Ross, Columbus, OH) during the first 2 days of life. In a manner consistent with the routines in the nursery, the infants were managed according to a minimal stimulation program, which included intubation, ventilation, and sedation (Table 1). All infants were clinically stable, had normal body temperature under radiant warmers, oxygen saturation of 95–98%, normal acid base status (pH 7.31 ± 0.01) (mean ± SE) and normal P_{CO_2} (36 ± 2 torr) on ventilatory settings described in Table 1. The infants received prophylactic antibiotics, but none had positive blood cultures or any clinical signs of septicemia. None of the infants had been fed, but they were all receiving TPN (Table 2). None of the infants received insulin during the course of the parenteral nutrition. The infants were studied on day 5.0 ± 0.1 of life (Table 1).

Procedures. As described in detail below, three independent methods of measuring gluconeogenesis were employed, one in each of three separate groups of infants: *1*) MIDA of circulating glucose during intravenous infusions of [U-^{13}C]glucose (35), *2*) MIDA of the appearance of ^{13}C in blood glucose during intravenous infusion of [2-^{13}C]glycerol (36,37), and *3*) deuterium incorporation in carbon 6 of glucose during infusion of ^2H_2O (38).

Tracers. [U-^{13}C]glucose (98+ atom% ^{13}C; 93.8% $^{13}C_6$), [2-^{13}C]glycerol (99 atom% ^{13}C), ^2H_2O (99.9 atom% ^{2}H), and [1-^{13}C]leucine (99 atom% ^{13}C) were purchased from Cambridge Isotope Laboratories (Andover, MA). The tracers were tested for sterility and pyrogenicity, dissolved in isotonic saline, and prepared for intravenous infusion by the research pharmacy at Texas Children's Hospital. The first eight infants were studied using [U-^{13}C]glucose. Subsequently, six infants were studied using [2-^{13}C]glycerol, and the last six were studied after administration of ^2H_2O.

TPN. Before the study, all infants received parenteral nutrition according to the established nursery practice policy as depicted in Table 2. Using this policy, glucose and amino acid infusion rates are increased rapidly over the first 3 days of life to reach the respective rates of 16.3 g · kg^{-1} · day^{-1} (11.3 mg · kg^{-1} · min^{-1}, 62 µmol · kg^{-1} · min^{-1}) and 3.1 g · kg^{-1} · day^{-1} (2.2 mg · kg^{-1} · min^{-1}), by the 3rd to 4th day of life. Lipids are introduced on day 4 at a rate of ~1 g · kg^{-1} · day^{-1} (0.7 mg · kg^{-1} · min^{-1}), and are increased to ~2 g · kg^{-1} · day^{-1} (1.4 mg · kg^{-1} · min^{-1}) on day 5 and ~3 g · kg^{-1} · day^{-1} (2.1 mg · kg^{-1} · min^{-1}) on day 6. For the 12- to 20-h period preceding the study period, all study infants received parenteral glucose at a dosage of 10.8 ± 0.2 mg · kg^{-1} · min^{-1} (60.0 ± 1.1 µmol · kg^{-1} · min^{-1}), amino acids at 2.2 ± 0.1 mg · kg^{-1} · min^{-1}, and lipids at 1.1 ± 0.2 mg · kg^{-1} · min^{-1} at constant rates.

At the onset of the study period, designated hour zero, stable isotopically labeled tracer infusions were begun as described in detail below. During each entire study period, the infants received amino acids (TrophAmine; McGaw, Irvine, CA) at a dosage of 2.2 ± 0.1 mg · kg^{-1} · min^{-1} and parenteral lipids (20% Intralipid; Baxter, Glendale, CA) at a dosage of 1.6 ± 0.1 mg · kg^{-1} · min^{-1}. Intralipid (20%) contains free glycerol, 22.5 mg/ml (244.6 µmol/ml), and triglycerides (soybean oil), 200 mg/ml. From its fatty acid composition (assuming that the triglycerides are completely hydrolyzed), the soybean oil provides 19.9 mg/ml (216.6 µmol/ml) of glycerol, resulting in a total glycerol supply of 42.4 mg/ml (461.2 µmol/ml) of Intralipid. At an infusion rate of 0.008 ± 0.001 ml · kg^{-1} · min^{-1}, Intralipid provides 0.32 ± 0.06 mg · kg^{-1} · min^{-1} (3.50 ± 0.66 µmol · kg^{-1} · min^{-1}) of glycerol.

For the 1st h of the study period, the glucose (dextrose; Abbott, Chicago) infusion rate was reduced stepwise from the prestudy infusion rate of 10.8 ± 0.2 mg · kg^{-1} · min^{-1} (60.0 ± 1.1 µmol · kg^{-1} · min^{-1}) to 6.1 ± 0.1 mg · kg^{-1} · min^{-1} (33.8 ± 0.2 µmol · kg^{-1} · min^{-1}) to minimize adaptive counterregulatory responses to a diminution in the supply of exogenous glucose. Thereafter, the glucose infusion was reduced further to the rate of 3.1 ± 0.1 mg · kg^{-1} · min^{-1} (17.1 ± 0.2 µmol · kg^{-1} · min^{-1}) for the remaining 7–11 h of the study period. This rate is significantly below the glucose turnover rates measured previously in both term and preterm infants (4,9–12).

Thus, at start of the study sampling period (4 h), the infants had received intravenous glucose at the rate of 3.1 ± 0.1 mg · kg^{-1} · min^{-1} (17.1 ± 0.2 µmol · kg^{-1} · min^{-1}) for 3 h, amino acids at the rate of 2.2 ± 0.1 mg · kg^{-1} · min^{-1} for 24 h and lipids at 1.6 ± 0.1 mg · kg^{-1} · min^{-1} for at least 4 h. These infusion rates were maintained for the remainder of the study period. There was no significant difference in the composition of the TPN solutions supplied to each of the three tracer method groups (glucose, 3.0–3.2 mg · kg^{-1} · min^{-1}; amino acids, 2.2–2.3 mg · kg^{-1} · min^{-1}; and lipids, 1.5–1.7 mg · kg^{-1} · min^{-1}).

Study design. Tracer and TPN solutions were administered via umbilical venous catheters, and blood samples were drawn from umbilical artery catheters already in place for clinical care purposes. The umbilical artery catheters were kept patent with isotonic saline both before and during the study period. The total blood volume required for this study was 2.2–2.5 ml, representing <3% of each subject's total blood volume.

To ensure that normoglycemia (blood glucose ≥2.2 mmol/l) was maintained, blood glucose concentrations were measured hourly at the bedside using a glucose analyzer (YSI 2300 Stat Plus; YSI, Yellow Springs, OH). Plasma insulin concentrations were measured at the onset and termination of the study using a conventional radioimmunoassay (RIA) (Linco, St Charles, MO). The detection limit for this RIA is 0.5 µU/ml. All isotopes were administered by Medinfusion 2010 infusion pumps (Medex, Duluth, GA).

TABLE 1
Subject characteristics, ventilator settings, and medications during the study period

Method	Subjects	Subject characteristics: Birth weight (g)	Gestational age (weeks)	Postnatal age (days)	Apgar score (at 5 min)	Ventilator settings: FiO_2	PIP/PEEP	Medications: Antenatal steroids	Phenobarbital doses (5 mg/kg)	Morphine dose (0.1 mg/kg)
[U-^{13}C]glucose										
	1	1,080	28	5	8	0.21	18/4	Yes	×1	×1
	2	800	26	5	8	0.21	18/5	No	×1	×2
	3	950	27	5	8	0.28	18/4	No	×1	×1
	4	860	25	6	8	0.21	18/5	Yes	×1	×1
	5	1,030	28	5	8	0.21	18/3	No	×1	×1
	6	990	27	6	8	0.27	26/5	No	—	—
	7	1,100	27	5	8	0.21	16/4	Yes	×1	×1
	8	880	27	5	9	0.21	20/5	Yes	×1	—
	Mean ± SE	961 ± 38	26.9 ± 0.4	5.3 ± 0.2	8	0.23 ± 0.01	19/5			
[2-^{13}C]glycerol										
	9	1,150	28	4	9	0.29	18/4	Yes	×1	×2
	10	1,050	28	5	6	0.21	18/4	Yes	×1	×1
	11	1,130	28	4	8	0.21	18/5	No	×1	—
	12	1,140	27	6	9	0.25	20/3	Yes	×1	—
	13	1,180	28	4	9	0.21	18/4	Yes	×1	—
	14	920	27	5	8	0.21	18/4	No	×1	×1
	Mean ± SE	1,095 ± 39	27.7 ± 0.2	4.7 ± 0.3	8	0.23 ± 0.01	18/4			
$^{2}H_2O$										
	15	1,070	29	5	9	0.25	20/4	Yes	×1	×1
	16	727	24	4	5	0.40	25/5	No	×1	×2
	17	1,060	27	4	9	0.21	16/5	No	×1	×2
	18	950	26	5	8	0.21	18/5	Yes	×1	×1
	19	900	26	5	8	0.30	18/5	No	—	—
	20	960	27	5	9	0.24	16/4	Yes	×1	—
	Mean ± SE	945 ± 51	26.5 ± 0.7	4.7 ± 0.2	8	0.27 ± 0.03	19/5			

FiO_2, fraction of oxygen in inspiratory gas; PIP, peak inspiratory pressure; PEEP, positive end-expiratory pressure.

MIDA during infusion of [U-^{13}C]glucose. At hour zero, a constant infusion of [U-^{13}C]glucose, metabolically equivalent to natural glucose, was begun at the rate of 16.7 ± 0.2 μmol · kg^{-1} · min^{-1} (3.0 ± 0.1 mg · kg^{-1} · min^{-1}) and was continued at this constant rate for 10 (four infants) to 12 h (four infants). During the 1st h of the infusion period, the infants received an additional 16.7 μmol · kg^{-1} · min^{-1} (3 mg · kg^{-1} · min^{-1}) of natural glucose as described above. Thereafter, [U-^{13}C]glucose represented the entire source of exogenous glucose. To estimate rates of proteolysis, [1-^{13}C]leucine was given at a constant rate of 0.140 ± 0.012 μmol · kg^{-1} · min^{-1} (0.019 ± 0.002 mg · kg^{-1} · min^{-1}) between study hours 4 and 8. Samples for blood glucose concentration and MIDA of plasma glucose and lactate as well as ^{13}C enrichment in plasma leucine and its intracellular transamination product, α-ketoisocaproic acid (KIC), as described below, were obtained immediately before starting the tracer infusion, and then at 2, 4, 6, 8, 10 and 12 h of tracer infusion.

MIDA during infusion of [2-^{13}C]glycerol. TPN was administered as described above (i.e., similar to the procedure used in the group studied by the [U-^{13}C]glucose method), except that the glucose supply was represented by natural glucose. Thus, during the 1st h of the infusion period, the infants received glucose at a rate of 33.3 μmol · kg^{-1} · min^{-1} (6 mg · kg^{-1} · min^{-1}) and then at 17.8 ± 0.6 μmol · kg^{-1} · min^{-1} (3.2 ± 0.2 mg · kg^{-1} · min^{-1}) for the remaining study period. At hour zero, an infusion of [2-^{13}C]glycerol was started at the constant rate of 3.87 ± 0.10 μmol · kg^{-1} · min^{-1} (0.36 ± 0.01 mg · kg^{-1} · min^{-1}) and was continued over 8 (one infant) to 10 h (five infants). To simultaneously measure the appearance rates (*Ra*) of plasma glucose and leucine, [U-^{13}C]glucose and [1-^{13}C]leucine were also infused at the rates of 0.54 μmol · kg^{-1} · min^{-1} (0.1 mg · kg^{-1} · min^{-1}) and 0.125 ± 0.004 μmol · kg^{-1} · min^{-1} (0.017 ± 0.001 mg · kg^{-1} · min^{-1}), respectively, during study hours 4–8. At this low infusion rate of [U-^{13}C]glucose, the fractions of singly and doubly labeled glucose molecules generated by the [U-^{13}C]glucose

TABLE 2
TPN administered routinely on days 1–4 of life, average study TPN administered during 8–12 h on day 5 of life, and total substrate delivery on day 5 of study

	Routine TPN: Day 1 (24 h)	Day 2 (24 h)	Day 3 (24 h)	Day 4 (24 h)	Study TPN (day 5 of life): Prestudy period (midnight to study start)	Study period (8–12 h)	Poststudy period (study end to midnight)	Total (day 5 of study)
Substrate (g/kg)								
Glucose	8–10	12.5–15	16.3	16.3	5.1 ± 0.1	2.0 ± 0.1	4.1 ± 0.1	11.1 ± 0.1
Amino acids*		2.4–2.9	3.1	3.1	1.0 ± 0.1	1.4 ± 0.1	0.8 ± 0.1	3.3 ± 0.1
Lipids†				1.0	0.5 ± 0.1	1.0 ± 0.1	0.6 ± 0.1	2.1 ± 0.1
Energy intake (kcal/kg)	27–34	51–61	67	76	26 ± 1	20 ± 1	22 ± 1	68 ± 1

Data are means ± SE or range. *TrophAmine; †Intralipid.

tracer were negligible and did not interfere with assessment of the ^{13}C appearance in blood glucose derived from [2-^{13}C]glycerol. Blood samples for measurement of substrate content and ^{13}C isotopic enrichments in blood glycerol, glucose, KIC, and leucine were obtained before the tracer infusion was started, and then at 4, 6, 8, and 10 h of infusion.

Incorporation of deuterium at carbon 6 of glucose after infusion of ^2H_2O. TPN was administered as described for the glycerol MIDA experiment above. Thus, during the 1st h of the infusion period, the infants received natural glucose at a rate of 33.3 μmol · kg^{-1} · min^{-1} (6 mg · kg^{-1} · min^{-1}) and then 17.1 ± 0.2 μmol · kg^{-1} · min^{-1} (3.1 ± 0.1 mg · kg^{-1} · min^{-1}) for the remaining study period. From hours 0–2 of the study, a total of 5 g/kg of sterile ^2H_2O (1.25 g/ml,made isotonic by the addition of sodium chloride) was administered intravenously at a constant rate of 0.033 ml · kg^{-1} · min^{-1}. To quantify the deuterium incorporation in carbon 6 of glucose, blood samples were drawn before start of the tracer infusion and then at study hours 4.5 and 5.5 in four infants and at study hours 8 and 10 in two infants. In the first four infants, the 5.5 h sample was followed by a 3-h infusion of [U-^{13}C]glucose (0.54 μmol · kg^{-1} · min^{-1} [0.1 mg · kg^{-1} · min^{-1}]) to measure plasma glucose *R*a from plasma [$^{13}C_6$]glucose enrichment in blood samples drawn at study hours 7 and 8. The last two infants did not receive [U-^{13}C]glucose.

Analyses

MIDA during infusion of [U-^{13}C]glucose. The pentaacetate derivative of glucose was prepared as described previously (32,33), and the positional distribution of [^{13}C]glucose isotopomers was determined by gas chromatography–mass spectrometry (GCMS) using a quadrupole instrument (HP 5989B; Hewlett-Packard, Palo Alto, CA) and a DB-1701 column (25 m × 0.25 mm × 0.25 μm) (J&W Scientific, Folsom, CA). Methane chemical ionization was used in the positive ion mode with selected monitoring of m/z 331–337, reflecting unlabeled glucose and glucose molecules labeled with ^{13}C in carbons 1–6.

The acetyl-pentafluorobenzyl derivative of lactate was prepared as described previously (50). In addition to the procedure described in this reference, the pentafluorobenzyl derivative was acetylated by addition of a mole excess of 2:1 acetic anhydride/pyridine (Aldrich, Milwaukee, WI). The ^{13}C enrichments in lactate carbons 1–3 were analyzed by GCMS (with the HP 5989B quadrupole instrument and an HP 5 column [25m × 0.25 mm × 0.25 μm]) using negative chemical ionization with methane as the reagent gas and selected monitoring of m/z 131–134.

After addition of an internal standard ([4, 5, 5, 5, 6, 6, 6-$^2H_7$]leucine [98 atom% ^2H, 86.5% $^2H_7$] [Merck, Sharp & Dohme, Montreal, Canada]), the oximeterbutyldimethylsilyl derivative of KIC and the heptafluorobutyric anhydride (HFBA) derivative of leucine were prepared as described below. Briefly, 50 μl of acidified plasma was applied to an AG 50-X8 cation-exchange column (100–200 mesh) (Bio-Rad, Hercules, CA). KIC was eluted by 0.01 mol/l HCl, and leucine by 5*N*-NH_4OH. The KIC eluate was made alkaline with 10*N*-NaOH, fresh 0.36 mol/l hydroxylamine hydrochloride (Fisher, Fair Lawn, NJ) was added, and the mixture was sonicated for 1 min and heated at 60°C for 30 min. After cooling, the samples were brought to pH <2 by addition of 6*N*-HCl, and KIC was extracted by ethyl acetate. Finally, after the extracted samples were dried under nitrogen, 50 μl *N*-methyl-*N*-(*t*-butyldimethylsilyl)trifluoroacetamide + 1% *t*-butyldimethylchlorosilane (Regis, Morton Grove, IL) was added, and the samples were kept tightly capped at room temperature for 24 h. After evaporation of the leucine eluate, the HFBA derivative was performed as described previously (51). The [$^{13}C_1$]KIC enrichment was analyzed by GCMS with the HP 5989B quadrupole instrument, using the electron impact mode and an HP 5 column (25 m × 0.25 mm × 0.25 μm), and the [$^{13}C_1$]leucine enrichment was analyzed on the same instrument and column, using negative chemical ionization with methane as the reagent gas. For KIC, m/z 316 and 317 were monitored to represent unlabeled KIC and [$^{13}C_1$]KIC, and for leucine, m/z 349 (unlabeled), 350 ($^{13}C_1$-labeled), and 356 were monitored to represent the heptadeuteroleucine internal standard.

MIDA during infusion of [2-^{13}C]glycerol. After addition of an internal standard, [1,1,2,3,3–$^2H_5$]glycerol (98 atom% ^2H, 93.5% $^2H_5$) (Cambridge Isotope) (32,33), the pentaacetate and triacetate derivatives of glucose and glycerol, respectively, were prepared and subsequently analyzed by GCMS using the HP 5989B quadrupole instrument and a DB-17 column (25 m × 0.25 mm × 0.25 μm). Methane positive chemical ionization was used with selected monitoring of m/z 331–337, reflecting unlabeled glucose (m/z 331), singly and doubly labeled glucose derived from [2-^{13}C]glycerol (m/z 332 and 333) and [$^{13}C_6$]glucose derived from [U-^{13}C]glucose (m/z 337). Ion intensities at the remaining isotopomer positions, m/z 334–336, were negligible. In addition, m/z 159, 160, and 164, reflecting unlabeled glycerol, $^{13}C_1$-labeled glycerol, and the pentadeuteroglycerol internal standard, were monitored. Plasma $^{13}C_1$ enrichments of KIC and leucine were analyzed as described above for the [U-^{13}C]glucose method.

Incorporation of deuterium at carbon 6 of glucose after infusion of ^2H_2O. Deuterium incorporation in carbon 6 of glucose was determined using the hexamethylenetetramine (HMT) derivative as described by Kalhan et al. (38). The deuterium enrichment in HMT was analyzed by GCMS, using an HP 5970 quadrupole instrument and an HP 5 column (25 m × 0.25 mm × 1.0 μm) in the electron impact mode with selected monitoring of m/z 140 and 141. HMT enrichments were converted to the corresponding glucose enrichments using a standard curve prepared from [1-$^2H_1$]glucose (99 atom% ^2H) (Cambridge Isotope) after conversion to sorbitol (39,52). Deuterium enrichment in body water (represented by plasma water) was measured by isotope ratio mass spectrometry (Finnigan Delta-E; Finnigan MAT, San Jose, CA) after reduction to hydrogen gas according to accepted methods (53,54). The [$^{13}C_6$]glucose enrichment during infusion of [U-^{13}C]glucose was analyzed as described above for the [2-^{13}C]glycerol method.

Calculations. The ion current ratios for glucose and lactate were normalized and expressed as mole fractions according to Fernandez et al. (55), using software provided by Dr. Henri Brunengraber (Case Western Reserve University, Cleveland, OH).

Plasma glucose *R*a was calculated from the $^{13}C_6$ enrichment of glucose (56,57): total glucose $Ra = (E_i/E_p) \times I$, where E_i is the $^{13}C_6$ enrichment of the infusate, E_p is the $^{13}C_6$ enrichment in plasma, and I is the infusion rate of [U-^{13}C]glucose (expressed in micromoles per kilogram per minute). The glucose production rate (GPR) was calculated as total glucose *R*a minus the exogenously infused glucose, which is the sum of all labeled and unlabeled glucose infused, i.e., [U-^{13}C]glucose plus natural glucose.

From the measured isotopic enrichments and mass isotopomer distribution patterns, gluconeogenesis as a fraction of glucose *R*a was calculated according to each of the respective methods: *1*) by MIDA during infusion of [U-^{13}C]glucose according to Tayek and Katz (35), *2*) by MIDA during infusion of [2-^{13}C]glycerol according to Hellerstein and Neese (36) and Neese et al. (37), and *3*) by quantifying incorporation of deuterium in the sixth carbon position of glucose after infusion of ^2H_2O according to Kalhan et al. (38) and Landau et al. (39). Total gluconeogenesis or gluconeogenic rate is reported as the product of the fractional gluconeogenic rate multiplied by the glucose *R*a. Gluconeogenesis as a fraction of the GPR represents the total gluconeogenic rate divided by the GPR. The gluconeogenic contribution from glycerol was calculated according to Hellerstein and Neese (37) using software provided by Drs. Marc Hellerstein and Richard Neese (University of California, San Francisco).

Plasma glycerol concentrations were calculated from the ion current ratio 159/164 using reverse isotope dilution principles relative to the labeled internal standard (32,33). Total plasma glycerol *R*a and endogenous glycerol *R*a were calculated as described above for glucose *R*a; likewise, the exogenous contribution included both the glycerol infused as tracer ([2-^{13}C]glycerol) and that derived from Intralipid (see above). Plasma leucine concentrations were calculated from the ion current ratio 323/316 using a standard curve based on standards with known mole ratios of the internal standard relative to natural leucine (58). Leucine *R*a was calculated from the plasma [$^{13}C_1$]KIC enrichment using the "reciprocal pool" tracer dilution technique (59,60).

Statistical analyses. All data are presented as means ± SE. Analysis of variance was used to test for differences among the three method groups as well as for effects of antenatal steroids on glucose and glycerol kinetics. Simple and multiple stepwise linear regression analyses were used to test for significant relationships between blood glucose, plasma insulin concentration, plasma glucose *R*a, GPR, gluconeogenic parameters, body weight, and gestational age.

RESULTS

Blood glucose concentrations measured during the study period are displayed in Fig. 1. At the start of the study period, plasma glucose concentrations were not statistically different among the groups and averaged 5.7 ± 0.7 mmol/l in the [U-^{13}C]glucose group (n = 8), 5.2 ± 0.4 mmol/l in the [2-^{13}C]glycerol group (n = 6), and 6.8 ± 1.1 mmol/l in the ^2H_2O group (n = 6). During the 1st 2 h of the study, plasma glucose concentrations declined as a consequence of the reduced exogenous glucose infusion rate, but from study hour 2 to the end of the study period, plasma glucose concentrations were constant, averaging 3.2 ± 0.2 mmol/l ([U-^{13}C]glucose group), 2.8 ± 0.2 mmol/l ([2-^{13}C]glycerol group), and 3.0 ± 0.1 mmol/l (^2H_2O group), and they did not differ statistically among the three method groups (Fig. 1 and Table 4).

Plasma insulin concentrations averaged 40 ± 6 μU/ml for the [U-^{13}C] glucose group and 27 ± 5 μU/ml for the [2-^{13}C]glycerol group at the onset of the study, and 10 ± 1 and 8 ± 2 μU/ml, respectively, at the termination of the study,

with values not statistically different between the two groups. Because of the relatively large amounts of plasma required for the HMT determination, insulin concentrations were measured in only a single infant in the ^2H_2O group at the onset of the study (46 µU/ml) and three infants at the termination of the study (9, 3, and 6 µU/ml), with values not obviously different from those in the other study groups. Plasma insulin concentration was directly related to blood glucose concentration ($r = 0.77$; $P < 0.001$).

Plasma glycerol concentration was measured only in the [2-^{13}C]glycerol study group and averaged 230 ± 7 µmol/l from the 4th h of study to its termination.

Plasma leucine concentration averaged 176 ± 18 µmol/l in the [U-^{13}C]glucose group, a value not significantly different from that determined in the [2-^{13}C]glycerol group, 188 ± 19 µmol/l. In two infants of the [U-^{13}C]glucose group (subjects 2 and 3), plasma leucine concentration and turnover rates could not be measured because of insufficient amounts of available plasma, and in the ^2H_2O group, [1-^{13}C]leucine was not infused because restriction on the blood sample volume that could be drawn precluded the determination of plasma leucine concentration and turnover rate.

Plasma isotopic enrichments of the individual glucose and lactate isotopomers used for MIDA calculations of gluconeogenesis (35–37) during infusion of [U-^{13}C]glucose and [2-^{13}C]glycerol are depicted in Table 3. In the infants in whom gluconeogenesis was studied using the [U-^{13}C]glucose tracer, the plasma [$^{13}C_6$]glucose enrichment averaged 57.9 ± 1.8% (coefficient of variation [CV] = 5.2 ± 1.4%) from the 4th h to the end of the study period. Thus, because both plasma glucose concentrations and isotopic enrichments were stable, the period from 4 h to the end of the study was designated as the steady-state period. Similarly, in the [2-^{13}C]glycerol and ^2H_2O tracer groups, in which small amounts of [U-^{13}C]glucose tracer were also infused, the plasma [$^{13}C_6$]glucose enrichments averaged 1.8 ± 0.1 and 2.0 ± 0.3%, at 4 and at 3 h, respectively.

In the [2-^{13}C]glycerol tracer group, plasma $^{13}C_1$-glycerol enrichment averaged 21.9 ± 1.6% (CV = 10.6 ± 1.0%) (Table 4). Likewise, in the ^2H_2O tracer study group, plasma water deuterium enrichment averaged 0.53 ± 0.04% (CV = 4.3 ± 2.1%) and deuterium enrichment in carbon 6 of glucose averaged 0.09 ± 0.02% (CV = 4.9 ± 1.2%). Thus, in each tracer infusion group, the isotopic steady state was achieved over the course of the measurement period.

As shown in Table 3, values for plasma glucose Ra were nearly identical in all three groups, averaging 27.6 ± 0.7 µmol · kg^{-1} · min^{-1} (5.0 ± 0.1 mg · kg^{-1} · min^{-1}) in the [U-^{13}C]glucose group (n = 8), 29.8 ± 1.9 µmol · kg^{-1} · min^{-1} (5.4 ± 0.3 mg · kg^{-1} · min^{-1}) in the [2-^{13}C]glycerol group (n = 6), and 28.6 ± 5.1 µmol · kg^{-1} · min^{-1} (5.2 ± 0.9 mg · kg^{-1} · min^{-1}) in the ^2H_2O group (n = 3). GPRs were also virtually identical: 10.7 ± 0.8 µmol · kg^{-1} · min^{-1} (1.9 ± 0.1 mg · kg^{-1} · min^{-1}), 11.3 ± 1.8 µmol · kg^{-1} · min^{-1} (2.0 ± 0.3 mg · kg^{-1} · min^{-1}), and 10.3 ± 5.2 µmol · kg^{-1} · min^{-1} (1.9 ± 0.9 mg · kg^{-1} · min^{-1}) for the [U-^{13}C]glucose, [2-^{13}C]glycerol, and ^2H_2O groups, respectively.

In the [2-^{13}C]glycerol tracer group, total plasma glycerol Ra (calculated from the [$^{13}C_1$]glycerol enrichment) averaged 17.9 ± 1.4 µmol · kg^{-1} · min^{-1} (1.7 ± 0.1 mg · kg^{-1} · min^{-1}), which is the endogenous glycerol Ra (averaging 10.5 ± 1.3 µmol · kg^{-1} · min^{-1} [1.0 ± 0.1 mg · kg^{-1} · min^{-1}]) plus the exogenous glycerol delivered via Intralipid (3.5 ± 0.7 µmol · kg^{-1} · min^{-1} [0.3 ± 0.1 mg · kg^{-1} · min^{-1}]; see METHODS) and via the glycerol tracer (3.9 ± 0.1 µmol · kg^{-1} · min^{-1} [0.4 ± 0.01 mg · kg^{-1} · min^{-1}]), totaling 7.4 ± 0.7 µmol · kg^{-1} · min^{-1} (0.7 ± 0.01 mg · kg^{-1} · min^{-1}). On average, 52 ± 5% of total glycerol Ra was converted to glucose (9.3 ± 0.4 µmol · kg^{-1} · min^{-1} [0.9 ± 0. 1 mg · kg^{-1} · min^{-1}]).

At the end of the 4-h [1-^{13}C]leucine infusion, the plasma [$^{13}C_1$]KIC enrichments were 2.2 ± 0.2 and 1.9 ± 0.1% in the [U-^{13}C]glucose and [2-^{13}C]glycerol groups, respectively. Total leucine Ra, calculated from these enrichments, averaged 6.2 ± 0.3 µmol · kg^{-1} · min^{-1} (0.8 ± 0. 1 mg · kg^{-1} · min^{-1}) (n = 6) and 6.3 ± 0.3 µmol · kg^{-1} · min^{-1} (0.8 ± 0. 1 mg · kg^{-1} · min^{-1}) (n = 6), in the two method groups, respectively. Leucine Ra from protein breakdown averaged 3.8 ± 0.3 µmol · kg^{-1} · min^{-1} (0.5 ± 0.1 mg · kg^{-1} · min^{-1}) in the [U-^{13}C]glucose study group and 3.9 ± 0.3 µmol · kg^{-1} · min^{-1} (0.5 ± 0. 1 mg · kg^{-1} · min^{-1}) in the [2-^{13}C]glycerol group.

Gluconeogenesis as a fraction of glucose Ra increased slightly from 4 to 10 h in the [U-^{13}C]glucose group, and it was nearly constant in the [2-^{13}C]glycerol group. However, neither the values obtained at 4 h nor those obtained at 8–10 h, nor the mean value from 4 h to the end of the study period, differed significantly between the two method groups. From 4 to 8–10 h of the study period, gluconeogenesis as a fraction of glucose Ra averaged 28 ± 2 and 26 ± 2% measured by [U-^{13}C]glucose and [2-^{13}C]glycerol, respectively (Table 4). Total gluconeogenesis (gluconeogenic rate) was 7.6 ± 0.6 and 7.6 ± 0.8 µmol · kg^{-1} · min^{-1} (1.4 ± 0.1 and 1.4 ± 0.1 mg · kg^{-1} · min^{-1}) (Fig. 2), and gluconeogenesis as a fraction of glucose production was 72 ± 5 and 73 ± 9%, for the [U-^{13}C]glucose and [2-^{13}C]glycerol groups, respectively (Table 4). Both glucose generated from glycerol and other substrates and glucose recycled via the Cori cycle are included in the values estimated by the [U-^{13}C]glucose and the [2-^{13}C]glycerol methods, but only the [2-^{13}C]glycerol method permits partitioning of the fractions contributed by the glycerol and nonglycerol components, 4.9 ± 0.7 and 2.7 ± 0.4 µmol · kg^{-1} · min^{-1} (0.9 ± 0.1 and 0.5 ± 0.1 mg · kg^{-1} · min^{-1}), respectively (Fig. 2). Thus, gluconeogenesis from glycerol contributed 64 ± 5% of total gluconeogenesis and accounted for 47 ± 8% of glucose production.

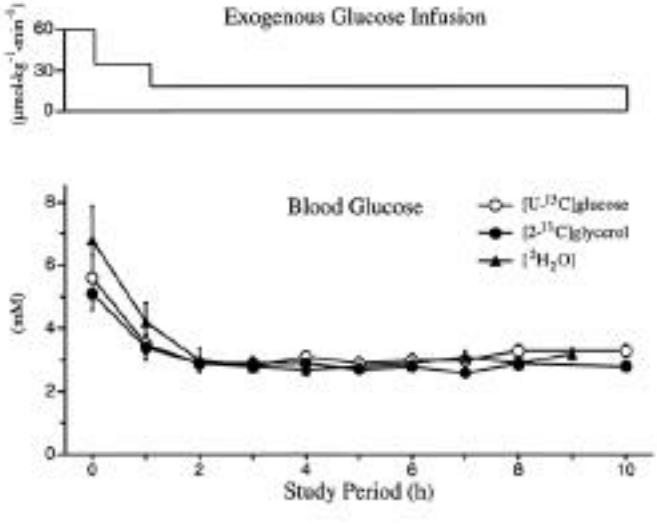

FIG. 1. Blood glucose concentrations during the study periods.

TABLE 3
Steady-state ^{13}C enrichments in glucose, glycerol, and lactate during infusion of [U-^{13}C]glucose and [2-^{13}C]glycerol

Enrichment	Method	
	[U-^{13}C]glucose* (%)	[2-^{13}C]glycerol† (%)
[$^{13}C_1$]glucose	4.24 ± 0.35	4.18 ± 0.48
[$^{13}C_2$]glucose	3.27 ± 0.30	1.02 ± 0.15
[$^{13}C_3$]glucose	3.57 ± 0.31	
[$^{13}C_4$]glucose	0.79 ± 0.07	
[$^{13}C_5$]glucose	0.47 ± 0.07	
[$^{13}C_6$]glucose	57.86 ± 1.81	
[$^{13}C_1$]lactate	5.33 ± 0.25	
[$^{13}C_2$]lactate	5.10 ± 0.22	
[$^{13}C_3$]lactate	30.46 ± 1.56	
[$^{13}C_1$]glycerol		21.88 ± 1.61

Data are means ± SE. *n = 8; †n = 6.

The ^2H_2O estimate of gluconeogenesis was significantly lower than those described above for the other two methods. In four infants, gluconeogenesis as a fraction of glucose Ra was determined at 4.5 and 5.5 h and averaged 8 ± 2%. This value is not significantly different from those obtained in two infants at 8 and 10 h, which averaged 9 ± 1% (i.e., both gluconeogenesis and equilibration between the hydrogens in body water and those attached to carbon 6 of glucose had reached steady state by 4.5 h). Thus, gluconeogenesis as a fraction of glucose Ra for the whole ^2H_2O group (n = 6) averaged 8 ± 1% (Table 3).

Glucose Ra, and thus GPR, could only be measured in three of these infants. Because the average glucose Ra and GPR in these infants (28.6 and 10.7 μmol · kg^{-1} · min^{-1}, respectively) did not differ from those of the other two groups (28.8 and 10.9 μmol · kg^{-1} · min^{-1}, respectively) (Table 4), they were considered representative for the whole ^2H_2O group. Therefore, the mean values for glucose Ra and GPR (28.6 and 10.7 μmol · kg^{-1} · min^{-1}, respectively) were used to calculate the gluconeogenic rates and gluconeogenesis as a fraction of GPR in the three infants in whom glucose Ra was not measured. Using these values, the gluconeogenic rate for the entire group of six infants studied by the ^2H_2O method averaged 2.3 ± 0.3 μmol · kg^{-1} · min^{-1} (0.4 ± 0.1 mg · kg^{-1} · min^{-1}). This value is not significantly different from the estimate of nonglycerol gluconeogenesis determined by the [2-^{13}C]glycerol tracer (2.7 μmol · kg^{-1} · min^{-1}). Adding the gluconeogenic contribution from glycerol (derived by [2-^{13}C]glycerol MIDA) to the nonglycerol estimate (measured by the ^2H_2O tracer) resulted in a total gluconeogenic rate of 7.2 ± 0.3 μmol · kg^{-1} · min^{-1} (1.3 ± 0.1 mg · kg^{-1} · min^{-1}) in the ^2H_2O method group, which is close to those measured by the [2-^{13}C]glycerol (7.6 μmol · kg^{-1} · min^{-1}) and the [U-^{13}C]glucose tracers (7.6 μmol · kg^{-1} · min^{-1}). Gluconeogenesis as a fraction of glucose production averaged 30.6 ± 10.8% for the whole ^2H_2O group (Table 4).

In infants exposed (n = 11) or not exposed (n = 9) to steroids antenatally (Table 1), glucose concentration averaged 3.0 ± 0.1 and 3.0 ± 0.1 mmol/l, glucose Ra was 29.7 ± 1.7 and 26.7 ± 1.0 μmol · kg^{-1} · min^{-1}, GPR was 12.0 ± 1.6 and 9.4 ± 1.3 μmol · kg^{-1} · min^{-1} and the gluconeogenic rate was 8.1 ± 0.6 and 6.9 ± 0.5 μmol · kg^{-1} · min^{-1}, respectively. Thus, in the present study,

TABLE 4
Average plasma glucose concentrations, plasma glucose Ra, GPR, and gluconeogenesis as fractions of glucose Ra and GPR between 4 and 8–10 h of study TPN

Method	Subjects	Glucose (mmol/l)	Glucose Ra (μmol · kg^{-1} · min^{-1})	GPR (μmol · kg^{-1} · min^{-1})	GNG fraction of Ra (%)	GNG fraction of GPR (%)
[U-^{13}C]glucose	1	3.7	28.5	11.8	33	80
	2	3.2	27.7	11.0	21	52
	3	2.7	25.6	7.8	24	80
	4	3.3	28.7	11.4	28	70
	5	3.9	29.7	13.0	24	55
	6	2.7	28.5	11.8	35	85
	7	2.8	28.6	11.9	31	74
	8	3.3	23.4	6.8	24	84
	Mean ± SE	3.2 ± 0.2	27.6 ± 0.7	10.7 ± 0.8	28 ± 2	72 ± 5
[2-^{13}C]glycerol	9	3.1	37.1	18.8	23	46
	10	2.3	32.7	9.9	31	100
	11	3.1	26.1	7.8	25	82
	12	2.8	27.9	10.3	33	88
	13	2.8	24.3	7.2	22	72
	14	2.8	30.8	13.6	21	47
	Mean ± SE	2.8 ± 0.1	29.8 ± 1.9	11.3 ± 1.8	26 ± 2	73 ± 9
^2H_2O	15	2.8	38.7	20.4	5	9
	16	2.9	22.7	3.3	12	82
	17	2.6	24.4	7.2	4	13
	18	3.0	—	—	11	—
	19	2.9	—	—	9	—
	20	3.5	—	—	9	—
	Mean ± SE	3.0 ± 0.1	—	—	8 ± 1	—

GNG, gluconeogenesis.

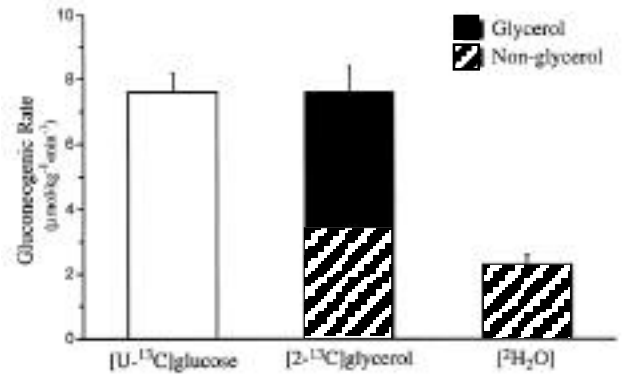

FIG. 2. Gluconeogenic rates measured by the [U-^{13}C]glucose, [2-^{13}C]glycerol, and $^{2}H_2O$ tracers. The $^{2}H_2O$ method measures only nonglycerol gluconeogenesis (see RESULTS).

none of these variables differed significantly between the infants born to mothers treated antenatally with steroids and those born to untreated mothers. However, the study was not designed to evaluate the impact of antenatal steroids.

DISCUSSION

It has only recently been possible to quantify gluconeogenesis in vivo (35–42,46), and the rates of gluconeogenesis from endogenous and exogenous precursors in VLBW infants are unknown. This study is the first to demonstrate that VLBW infants receiving an adequate parenteral supply of amino acids and lipids maintain stable and normal blood glucose concentrations for a prolonged period (8–12 h) when glucose is supplied at half the normal glucose turnover rate (4,9–12). Under these conditions, values for total plasma glucose *R*a were similar to those reported previously for newborn infants (4,9–12), and gluconeogenesis was the principal source of hepatic glucose output, apparently accounting for ~70% of the infant's glucose production. Glycerol contributed ~60% of total gluconeogenesis and nearly half of hepatic glucose output, compared with 5–20% in fasting newborns studied previously (29–33). Thus, in our infants, glycerol was the principal gluconeogenic substrate. As one might anticipate, total glycerol *R*a, which includes exogenously infused glycerol, was higher than that previously reported in newborns (29–33). However, the values for glycerol *R*a from endogenous sources (~11 μmol · kg^{-1} · min^{-1}) were only marginally higher than those reported in extremely premature infants receiving glucose at low rates but no intravenous lipids or amino acids (~8 μmol · kg^{-1} · min^{-1}) (33). They were also similar to values found in fasting preterm and term newborns (9–12 μmol · kg^{-1} · min^{-1}) (30–32), with the exception of data from a single study in which glycerol *R*a was only 4.4 μmol · kg^{-1} · min^{-1} (29) when measured 2–4 h after oral feeding in much larger infants than those studied in the present work. Thus, under the conditions of this study, the administration of parenteral nutrients did not inhibit the hydrolysis of endogenous triglycerides.

Our leucine kinetic data (total leucine *R*a, and rates of leucine derived from proteolysis) are similar to those obtained by others in newborn infants receiving similar amounts of amino acids and lipids but more glucose (61–63). Because of the study design, it was not possible to measure leucine oxidation rates. However, we calculated the oxidation rates that would have been required to produce a zero protein balance (protein synthesis = protein breakdown). In the [U-^{13}C]glucose and [2-^{13}C]glycerol study groups, the calculated leucine oxidation rates required to reduce protein balance to zero are 2.42 ± 0.03 and 2.43 ± 0.02 μmol · kg^{-1} · min^{-1}, respectively. These values would correspond to 40 ± 2 and 39 ± 2% of total leucine *R*a, in the two groups, respectively, values that are higher than those demonstrated in previous studies of newborn infants receiving TPN (61,63–65). Because actual leucine oxidation rates in our infants were presumably similar to those previously published (i.e., <40%) (61,63–65), and the rates of proteolysis were very close to those previously reported, it is unlikely that the infants of our study could have maintained normoglycemia at the expense of protein catabolism. In any event, our subjects were receiving appropriate quantities of energy and other macronutrients, and it is plausible to presume that their energy balance was truly positive.

Because each of the three recently described stable isotope methods of measuring gluconeogenesis has its own strengths and weaknesses (35–42), and the acceptable domains of validity of each have been debated (42–46), an important objective of the present study was to compare all three methods in premature infants studied under identical conditions.

Measuring gluconeogenesis by [U-^{13}C]glucose (35) and MIDA accounts for label dilution and exchange at the levels of both pyruvate/Krebs cycle and the triose phosphates. The equations used to calculate gluconeogenesis using [U-^{13}C]glucose and MIDA as described by Tayek and Katz (35) have recently been challenged by Landau et al. (43), who provided "corrected" equations. However, in a recent publication, Katz and Tayek (42) demonstrate that calculating gluconeogenesis using their own "uncorrected" equations in 40-h–fasted human adults results in values that agree with those obtained by Landau et al. (40), using deuterium oxide with assessment of the deuterium enrichment at glucose carbon 5, and by Hellerstein et al. (41), using [2-^{13}C]glycerol and MIDA. Thus, when applied to 65-h–fasted adults, the "corrected" equations of Landau et al. (43) resulted in much lower calculated estimates of gluconeogenesis (~33%) than *1*) the 92% obtained by Katz and Tayek (42) in 40-h–fasted adults, using the same equations used in the present study; *2*) the 94% obtained by Landau et al. (40) in 65-h–fasted adults, using deuterium oxide and deuterium incorporation at glucose carbon 5; and *3*) the 80% found by Hellerstein et al. (41) in 60-h–fasted adults, using [2-^{13}C]glycerol tracer and MIDA analysis. Furthermore, the estimate provided by Landau's "corrected" equations (43) was also considerably lower than the 71% obtained using deuterium oxide and deuterium incorporation at glucose carbon 6 (40), an approach that is known to underestimate gluconeogenesis (see below).

Landau and co-workers (43) reported that the values obtained by the "corrected" equations are unreasonably low and suggest that this underestimation of gluconeogenesis is due to *1*) the failure of the [U-^{13}C]glucose method to include the contributions of glycerol and perhaps some amino acids, and *2*) the fact that peripheral lactate measurements may not necessarily reflect the intrahepatic distribution of label. However, Landau et al. (66) have demonstrated that the infusion of [U-^{13}C]glycerol resulted not only in a triply labeled glucose molecule, but in singly and doubly labeled glucose

molecules as well. This outcome was most likely a result of labeled glycerol molecules being incorporated into glucose and then recycled back to glucose via pyruvate, or of glycerol going directly to pyruvate via glycolysis and returning to glucose via gluconeogenesis. In addition, they have demonstrated that during infusion of [2-^{14}C]glycerol, ^{14}C appeared not only in the 2 and 5 positions of glucose, which would be expected from a direct conversion of glycerol to glucose, but in the 1 and 6 positions as well, which could occur only via pyruvate, presumably through the secondary metabolism of the labeled glucose molecule (66).

Little information is available regarding amino acids, and we cannot speculate on their unique contribution. However, the two major gluconeogenic amino acids, alanine and glutamine, are in large measure synthesized from carbon derived from the metabolism of glucose in muscle and are then routed back into glucose by entering the gluconeogenic pathway via pyruvate going to oxaloacetate and via α-ketoglutarate going to oxaloacetate, respectively. The peripheral lactate measurements may not necessarily reflect intrahepatic lactate values. However, Landau et al. (44) have demonstrated that lactate enrichment values obtained in the brachial artery and the renal and hepatic veins were virtually identical.

In addition, the application of the "corrected" equations to our data would have resulted in a fractional gluconeogenesis of 15%, which is half the value obtained by the [2-^{13}C]glycerol tracer and double that of the deuterium oxide method. Such an outcome is incompatible with the theoretical bases for each method. Because the [U-^{13}C]glucose and [2-^{13}C]glycerol tracers both measure total gluconeogenesis, they should provide equal results, as was the case in the present study when Katz's uncorrected equations were applied to our data.

The [2-^{13}C]glycerol tracer measures gluconeogenesis from the triose phosphates, which represent a more immediate precursor of glucose. However, because the triose phosphate pool enrichment cannot be measured directly, it is calculated by MIDA from the relationship between glucose molecules that have been singly and doubly labeled with ^{13}C derived from [2-^{13}C]glycerol (36,37). The accuracy of the [2-^{13}C]glycerol MIDA has been questioned, largely because the potential zonation of glycerol release from the liver may result in inhomogeneity of the precursor pool (44,45). Because preterm infants have considerably higher glycerol turnover rates than adults (33,67), the potential zonation problem is likely minimized. The [2-^{13}C]glycerol method has been validated by Peroni et al. (46) as well as Hellerstein et al. (41), suggesting that this method gives accurate estimates of gluconeogenesis, provided that the glycerol tracer is infused at rates sufficient to generate precise measurements of the fractions of singly and doubly labeled glucose molecules (41,46). As is apparent from Table 4, this criterion was fulfilled in the present study. The requirement of high tracer infusion rates is a potential disadvantage in that the mass of the tracer itself may perturb glucose metabolism. We do not believe this is a significant problem in the present study because *1*) the infusion rate of the glycerol tracer corresponded to ~20% of the glycerol turnover rate, and *2*) the purpose of the study was to determine glucose kinetics during parenteral infusion of noncarbohydrate nutrients given at rates sufficient to maintain daily energy balance.

The deuterium oxide method provides the most straightforward approach to measuring gluconeogenesis. However, measurements based on deuterium incorporation into glucose carbon 6 alone underestimate gluconeogenesis, because the gluconeogenic contribution from glycerol is not included and the equilibration between body water and carbon 6 of glucose is incomplete (39,40). These problems can be solved by measuring deuterium incorporation in glucose carbon 5 (40). However, this approach requires large-volume blood samples, eliminating its practical application in premature infants.

Because of the higher glycerol *R*a in newborns compared with adults (29–33,67), a factor that minimizes problems with the glycerol method, the present study provided a unique opportunity to test the three methodologies. Our results demonstrate that the [U-^{13}C]glucose and [2-^{13}C]glycerol methods provided almost identical estimates of gluconeogenesis and that, as expected, the deuterium oxide estimate was significantly lower. It is noteworthy, however, that when the contribution from glycerol (measured by the [2-^{13}C]glycerol tracer) was added to the deuterium oxide estimate, all three methods provided remarkably similar estimates of gluconeogenesis. Thus, our results agree with those described in independently published studies in long-fasted adults in which the [U-^{13}C]glucose, [2-^{13}C]glycerol, and deuterium oxide–carbon 5 methods (all of which include the contribution from glycerol) provided similar estimates of gluconeogenesis (40–42), each significantly higher than the deuterium oxide–carbon 6 estimate (39,40). However, it should be noted that the deuterium oxide–carbon 6 estimate differed more from the others in our premature infants than it did in the adults, presumably as a consequence of either the larger contribution from glycerol in the infants (29–33,40,67) or less complete equilibration between ^{2}H in body water and glucose carbon 6, although the latter appears unlikely, given the rapid substrate flux and water turnover rates observed in preterm infants.

Thus, our data imply that in premature infants, the main difference between the [U-^{13}C]glucose and [2-^{13}C]glycerol estimates of gluconeogenesis on one hand and the deuterium oxide-carbon 6 estimate on the other is accounted for by gluconeogenesis from glycerol.

In summary, the results of this study suggest that when the supply of exogenous glucose is reduced to about half the glucose turnover rate, VLBW infants maintain normoglycemia via the combined contributions of glycogenolysis and gluconeogenesis if adequate amounts of parenterally delivered lipids and amino acids are also supplied simultaneously. During lipid infusion, after ~4 h of reduced delivery of exogenous glucose, gluconeogenesis becomes the principal source of glucose production, and glycerol becomes the primary gluconeogenic substrate. These findings demonstrate that key enzymes regulating gluconeogenesis are not rate limiting for glucose production in VLBW infants. Under identical conditions, the three methods provide similar results when the contribution from glycerol is added to the deuterium oxide estimate.

Because our results demonstrate that VLBW infants can use noncarbohydrate carbon sources to produce glucose, they also imply that it may be possible to substitute noncarbohydrate substrates for part of the exogenous glucose given routinely, thereby diminishing the risk of hyperglycemia without increasing the risk of hypoglycemia, energy deficiency, or protein catabolism.

ACKNOWLEDGMENTS

This work is a publication of the U.S. Department of Agriculture/Agricultural Research Service Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, Texas. This project was supported by grants from U.S. Department of Agriculture Cooperative Agreement 58-6250-6-001 and the General Clinical Research Center, National Center for Research Resources, National Institutes of Health Grant MO1-RR-001888. The contents of this publication do not necessarily reflect the views or policies of the U.S. Department of Agriculture, nor does mention of trade names, commercial products, or organizations imply endorsement from the U.S. government.

We would like to thank the research nurses Pamela Burns, Leanne Renfro, Ellen Newton, Cindy Bryant, and Linda Peas'ant for excellent assistance, the pharmacists at Texas Children's Hospital for valuable help in mixing the TPN and isotope solutions, the staff of the neonatal intensive care unit for professional collaboration, Dr. William W. Wong (Children's Nutrition Research Center, Houston, TX) for isotope ratio mass spectrometry analyses of body water, Dr. Satish C. Kalhan (Case Western Reserve University, Cleveland, OH) for sharing with us his experience with the ^2H_2O method, Dr. Henri Brunengraber (Case Western Reserve University, Cleveland, OH) for kindly providing software to normalize the ion current ratios for glucose and lactate, and Drs. Marc Hellerstein and Richard Neese (University of California, San Francisco) for providing us with the procedures for the [2-^{13}C]glycerol MIDA as well as the software to calculate gluconeogenesis using this method.

REFERENCES

1. Guyer B, Martin JA, MacDorman MF, Andersson RN, Strobino DM: Annual summary of vital statistics—1996. *Pediatrics* 100:905–918, 1997
2. Siesjo BK: Hypoglycemia, brain metabolism, and brain damage. *Diabetes Metab Rev* 4:113–144, 1988
3. Gruenwald P, Minh HN: Evaluation of body and organ weights in perinatal pathology: I. Normal standards derived from autopsies. *Am J Clin Path* 34:247–253, 1960
4. Bier DM, Leake RD, Haymond MW, Arnold KJ, Gruenke LD, Sperling MA, Kipnis DM: Measurement of "true" glucose production rates in infancy and childhood with 6,6-dideuteroglucose. *Diabetes* 26:1016–1023, 1977
5. Shelley HJ: Glycogen reserves and their changes at birth and in anoxia. *Brit Med Bull* 17:137–143, 1961
6. Widdowson EM, Spray CM: Chemical development in utero. *Arch Dis Child* 26:205–214, 1951
7. Darmaun D, Haymond MW, Bier DM: Metabolic aspects of fuel homeostasis in the fetus and neonate. In *Endocrinology*. 3rd ed. Vol. 3. De Groot LJ, Ed. New York, Grune and Stratton, 1993, p. 2258–2286
8. Sauer PJ, Dane HJ, Visser HK: Longitudinal studies on metabolic rate, heat loss, and energy cost of growth in low birth weight infants. *Pediatr Res* 18:254–259, 1984
9. Sunehag A, Ewald U, Larsson A, Gustafsson J: Glucose production rate in extremely immature neonates (<28 w) studied by use of deuterated glucose. *Pediatr Res* 33:97–100, 1993
10. Sunehag A, Gustafsson J, Ewald U: Very immature infants (≤30 weeks) respond to glucose infusion with incomplete suppression of glucose production. *Pediatr Res* 36:550–557, 1994
11. Tyrala EE, Chen X, Boden G: Glucose metabolism in the infant weighing less than 1100 grams. *J Pediatr* 125:283–287, 1994
12. Kalhan SC, Savin SM, Adam PAJ: Estimation of glucose turnover with stable tracer glucose-1-^{13}C. *J Lab Clin Med* 89:285–294, 1977
13. Ziegler EE, O'Donnell AM, Nelson SE, Fomon SI: Body composition of the reference fetus. *Growth* 40:329–341, 1976
14. Brion LP, Fleischman AR, McCarton C, Schwarz GJ: A simple estimate of glomerular filtration rate in low birth weight infants during the first year of life: noninvasive assessment of body composition and growth. *J Pediatr* 109:698–707, 1986
15. Sheng H-P, Nichols BL Jr: Body composition of the neonate. In *Principles of Perinatal-Neonatal Metabolism*. Cowett RM, Ed. New York, Springer-Verlag, 1991, p. 650–670
16. Santalucia T, Camps M, Munoz P, Testar X, Palacin M, Zorzano A: Developmental regulation of Glut-1 (erythroid/Hep G2) and Glut-4 (muscle/fat) glucose transporter expression in rat heart, skeletal muscle, and brown adipose tissue. *Endocrinology* 130:837–846, 1992
17. Hughes SJ: The role of reduced glucose transporter content and glucose metabolism in the immature secretory responses of fetal rat pancreatic islets. *Diabetologia* 37:134–140, 1994
18. Grasso S, Distefano G, Messina A, Vigo R, Reitano G: Effect of glucose priming on insulin response in the premature infant. *Diabetes* 24:291–294, 1975
19. Ktorza A, Bihoreau M-T, Nurjhan N, Picon L, Girard J: Insulin and glucagon during the perinatal period: secretion and metabolic effects on the liver. *Biol Neonate* 48:204–220, 1985
20. Reitano G, Grasso S, Distefano G, Messina A: The serum insulin and growth hormone response to arginine and arginine with glucose in the premature infant. *J Clin Endocrinol Metab* 33:924–928, 1971
21. Ogata ES: Carbohydrate metabolism in the fetus and neonate and altered neonatal glucoregulation. *Ped Clin North Am* 33:25–45, 1986
22. Pollak A, Cowett RM, Schwartz R, Oh W: Glucose disposal in low-birth-weight infants during steady state hyperglycemia: effects of exogenous insulin administration. *Pediatrics* 61:546–549, 1978
23. Lilien LD, Rosenfield RL, Baccaro MM, Pildes RS: Hyperglycemia in stressed small premature neonates. *J Pediatr* 94:454–459, 1979
24. Dweck HS, Cassady G: Glucose intolerance in infants of very low birth weight. I. Incidence of hyperglycemia in infants of birth weights 1100 grams or less. *Pediatrics* 53:189–195, 1974
25. Cowett RM, Oh W, Pollak A, Schwartz R, Stonestreet BS: Glucose disposal of low birth weight infants: steady state hyperglycemia produced by constant intravenous glucose infusion. *Pediatrics* 63:389–396, 1979
26. Cowett RM, Oh W, Schwartz R: Persistent glucose production during glucose infusion in the neonate. *J Clin Invest* 71:467–475, 1983
27. Kalhan SC, Oliven A, King KC, Lucero C: Role of glucose in the regulation of endogenous glucose production in the human newborn. *Pediatr Res* 20:49–52, 1986
28. Van Aerde JEE, Sauer PJJ, Pencharz PB, Smith JM, Swyer PR: Effect of replacing glucose with lipid on the energy metabolism of newborn infants. *Clin Sci* 76:581–588, 1989
29. Bougnères PF, Karl IE, Hillman LS, Bier DM: Lipid transport in the human newborn: palmitate and glycerol turnover and the contribution of glycerol to neonatal hepatic glucose output. *J Clin Invest* 70:262–270, 1982
30. Patel D, Kalhan S: Glycerol metabolism and triglyceride–fatty acid cycling in the newborn: effects of maternal diabetes and intrauterine growth retardation. *Pediatr Res* 31:52–58, 1992
31. Sunehag A, Gustafsson J, Ewald U: Glycerol carbon contributes to hepatic glucose production during the first eight hours in healthy term infants. *Acta Paediatr* 85:1339–1343, 1996
32. Fjeld CR, Cole FS, Bier DM: Energy expenditure, lipolysis, and glucose production in preterm infants treated with theophylline. *Pediatr Res* 32:693–698, 1992
33. Sunehag A, Ewald U, Gustafsson J: Extremely preterm infants (<28 weeks) are capable of gluconeogenesis from glycerol on their first day of life. *Pediatr Res* 36:550–557, 1996
34. Frazer TE, Karl IE, Hillman LS, Bier DM: Direct measurement of gluconeogenesis from [2,3-$^{13}C_2$]alanine in the human neonate. *Am J Physiol* 240:E615–E621, 1981
35. Tayek JA, Katz J: Glucose production, recycling, Cori cycle, and gluconeogenesis in humans: relation to serum cortisol. *Am J Physiol* 272:E476–E484, 1997
36. Hellerstein MK, Neese RA: Mass isotopomer distribution analysis: a technique for measuring biosynthesis and turnover of polymers. *Am J Physiol* 263:E988–E1001, 1992
37. Neese RA, Schwartz J-M, Faix D, Turner S, Letscher A, Vu D, Hellerstein MK: Gluconeogenesis and intrahepatic triose phosphate flux in response to fasting or substrate loads. *J Biol Chem* 270:14452–14466, 1995
38. Kalhan SC, Trivedi R, Singh S, Chandramouli V, Schumann WC, Landau BR: A micromethod for the measurement of deuterium bound to carbon-6 of glucose to quantify gluconeogenesis in vivo. *J Mass Spectrom* 30:1588–1592, 1995
39. Landau BR, Wahren J, Chandramouli V, Schumann WC, Ekberg K, Kalhan SC: Use of ^2H_2O for estimating rates of gluconeogenesis. *J Clin Invest* 95:172–178, 1995
40. Landau BR, Wahren J, Chandramouli V, Schumann WC, Ekberg K, Kalhan SC: Contributions of gluconeogenesis to glucose production in the fasted state. *J Clin Invest* 98:378–385, 1996
41. Hellerstein MK, Neese RA, Linfoot P, Christiansen M, Turner S, Letscher A: Hepatic gluconeogenic fluxes and glycogen turnover during fasting in

humans: a stable isotope study. *J Clin Invest* 100:1305–1319, 1997
42. Katz J, Tayek JA: Gluconeogenesis and Cori cycle in 12, 20 and 40-hour fasted humans. *Am J Physiol* 275:E537–E542, 1998
43. Landau BR, Wahren J, Ekberg K, Previs SF, Yang D, Brunengraber H: Limitations in estimating gluconeogenesis and Cori cycle from mass isotopomer distributions using [U-$^{13}C_6$]glucose. *Am J Physiol* 274:E954–E961, 1998
44. Landau BR, Fernandez CA, Previs SF, Ekberg K, Chandramouli V, Wahren J, Kalhan SC, Brunengraber H: A limitation in the use of mass isotopomer distributions to measure gluconeogenesis in fasting adults. *Am J Physiol* 269:E18–E26, 1995
45. Previs SF, Fernandez CA, Yang D, Soloviev MV, David F, Brunengraber H: Limitations of the mass isotopomer distribution analysis of glucose to study gluconeogenesis. *J Biol Chem* 270:19806–19815, 1995
46. Peroni O, Large V, Beylot M: Measuring gluconeogenesis with [2-^{13}C]glycerol and mass isotopomer distribution analysis of glucose. *Am J Physiol* 269:E516–E523, 1995
47. Keshen T, Miller R, Jahoor F, Jacsic T, Reeds PJ: Glucose production and gluconeogenesis are negatively related to body weight in mechanically ventilated very low birth weight neonates. *Pediatr Res* 41:132–138, 1997
48. Lubchenco LO, Hansman C, Dessler M, Boyd E: Intrauterine growth as estimated from liveborn birth-weight data at 24 to 42 weeks of gestation. *Pediatrics* 32:793–800, 1963
49. Ballard JL, Khoury JC, Wedig K, Wang L, Eilers-Walsman BL, Lipp R: New Ballard score, expanded to include extremely premature infants. *J Pediatr* 119:417–423, 1991
50. Hachey DL, Patterson BW, Reeds PJ, Elsas LJ: Isotopic determination of organic keto acid pentafluorobenzyl esters in biological fluids by negative chemical ionization gas chromatography/mass spectrometry. *Anal Chem* 63:919–923, 1991
51. Patterson BW, Hachey DL, Cook GL, Amann JM, Klein PD: Incorporation of a stable isotopically labeled amino acid into multiple human apolipoproteins. *J Lipid Res* 32:1063–1072, 1991
52. Muntz JA, Carrol RE: A method for converting glucose to fructose. *J Biol Chem* 235:1258–1260, 1960
53. Wong WW, Lee LS, Klein PD: Deuterium and oxygen-18 measurements on microliter samples of urine, plasma, saliva, and human milk. *Am J Clin Nutr* 45:905–913, 1987
54. Wong WW, Clarke LL, Llaurador M, Klein PD: A new zinc product for the reduction of water in physiological fluids to hydrogen gas for $^2H/^1H$ isotope ratio measurements. *Eur J Clin Nutr* 46:69–71, 1992
55. Fernandez CA, Des Rosiers C, Previs SF, David F, Brunengraber H: Correction of 13C mass isotopomer distributions for natural stable isotope abundance. *J Mass Spectrom* 31:255–262, 1996
56. Bier DM, Arnold KJ, Sherman WR, Holland WH, Holmes WF, Kipnis DM: In vivo measurement of glucose and alanine metabolism with stable isotope tracers. *Diabetes* 26:1005–1015, 1977
57. Bougnères PF: Stable isotope tracers and the determination of fuel fluxes in newborn infants. *Biol Neonate* 52:87–96, 1987
58. Haymond MW, Howard CP, Miles JM, Gerich JE: Determination of leucine flux in vivo by gas chromatography–mass spectrometry utilizing stable isotopes for trace and internal standard. *J Chrom* 183:403–409, 1980
59. Matthews DE, Motil KJ, Rohrbaugh DK, Burke JF, Young VR, Bier DM: Measurement of leucine metabolism in man from a primed, continuous infusion of L-[1-^{13}C]leucine. *Am J Physiol* 238:E473–E479, 1980
60. Schwenk WF, Beaufrere B, Haymond MW: Use of reciprocal pool specific activities to model leucine metabolism in humans. *Am J Physiol* 249: E646–E650, 1985
61. Poindexter BB, Karn CA, Ahlrichs JA, Wang J, Leitch CA, Liechty EA, Denne SC: Amino acids suppress proteolysis independent of insulin throughout the neonatal period. *Am J Physiol* 272:E592–E599, 1997
62. Denne SC, Karn CA, Ahlrichs JA, Dorotheo AR, Wang J, Liechty EA: Proteolysis and phenylalanine hydroxylation in response to parenteral nutrition in extremely premature and normal newborns. *J Clin Invest* 97:746–754, 1996
63. Battista MA, Price PT, Kalhan SC: Effect of parenteral amino acids on leucine and urea kinetics in preterm infants. *J Pediatr* 128:130–134, 1996
64. Rivera A Jr, Bell EF, Bier DM: Effect of intravenous amino acids on protein metabolism of preterm infants during the first three days of life. *Pediatr Res* 33:106–111, 1993
65. Denne SC, Karn CA, Wang J, Liechty JA: Effect of intravenous glucose and lipid on proteolysis and glucose production in normal newborns. *Am J Physiol* 269:E361–E367, 1995
66. Landau BR, Wahren J, Previs SF, Ekberg K, Chandramouli V, Brunengraber H: Glycerol production and utilization in humans: sites and quantitation. *Am J Physiol* 271:E1110–E1117, 1996
67. Bortz MW, Paul P, Haff AC, Holmes WL: Glycerol turnover and oxidation in man. *J Clin Invest* 51:1537–1546, 1972