

Glycerol Metabolism and Triglyceride-Fatty Acid Cycling in the Human Newborn: Effect of Maternal Diabetes and Intrauterine Growth Retardation

DEEPAK PATEL AND SATISH KALHAN

Department of Pediatrics, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106

ABSTRACT. Kinetics of glycerol metabolism and triglyceride/fatty acid cycling were quantified in 12 healthy, normal, appropriate-for-gestational-age (AGA) infants, eight small-for-gestational-age (SGA) infants, and five infants of insulin-dependent diabetic mothers (IDM) at less than 48 h of age. Stable isotope-labeled [$2\text{-}^{13}\text{C}$]glycerol and [$6,6\text{-}^2\text{H}_2$]glucose in combination with indirect respiratory calorimetry were used. The tracers were used as constant rate infusion and steady state isotopic enrichment of glucose, glycerol, and bicarbonate was measured by mass spectrometric methods. After a 7- to 9-h fast, the plasma glucose, glycerol, and FFA concentrations were similar in the AGA and IDM groups. In the SGA group, the plasma glucose concentration was significantly lower than that in the AGA group throughout the study, but plasma FFA and glycerol concentrations were not different from those in the AGA infants. Plasma betahydroxybutyrate concentration was significantly elevated in the AGA group compared with IDM and SGA infants (AGA 0.59 ± 0.39 , SGA 0.35 ± 0.09 , IDM 0.33 ± 0.21 mmol/L; mean \pm SD). The rate of appearance of glycerol was significantly elevated ($p < 0.05$) in SGA infants (AGA 9.47 ± 2.11 , IDM 9.55 ± 2.14 , SGA 12.15 ± 3.87 $\mu\text{mol/kg}\cdot\text{min}$). Between 80 and 90% of glycerol turnover was converted to glucose, accounting for 20% of glucose turnover with no significant difference in the three groups. Approximately 35% of glycerol carbon was recovered in the bicarbonate (CO_2) pool. Less than 5% of CO_2 carbon was derived from glycerol. Estimation of triglyceride-fatty acid cycle revealed that the triglyceride energy mobilized was increased in SGA infants. Only 22–24% of the triglyceride energy released was oxidized to CO_2 in the newborn infants; the majority (76–78%) was recycled back to the adipose tissue. These data show that lipolysis is active in the immediate neonatal period. The contribution of fat to oxidative metabolism is increased in SGA infants. The major metabolic fate of glycerol in the neonate is conversion to glucose, and glycerol is a minor contributor to oxidative metabolism. (*Pediatr Res* 31: 52–58, 1992)

GC-MS, gas chromatograph-mass spectrometer
 $\dot{V}\text{O}_2$, O_2 consumption
 $\dot{V}\text{CO}_2$, CO_2 production
Ra, rate of appearance

The process of birth constitutes the transition of the fetus, receiving its nutrient supply from the mother, to the newborn, deriving its energy and nutrient supply from intermittent feeding and endogenous sources. During the immediate postnatal period, there is a rapid decrease in plasma glucose concentration along with an increase in FFA and glycerol (1–3). The latter, representing an initiation of lipolysis in the adipose tissue, is associated with a shift in fuel source for energy from predominantly carbohydrates *in utero* to a carbohydrate and fat fuel mix in the extrauterine life (4, 5). Such a shift is also evidenced by the change in RQ, which declines to approximately 0.8 by 2 h of age, suggesting a significant contribution of fat to the oxidative metabolism (5–7). Within the adipose tissue, during lipolysis, triglycerides are hydrolyzed, resulting in release of FFA and glycerol. Although FFA may be reesterified locally in the adipose tissue, glycerol once released cannot be reused locally to synthesize triglycerides because of the absence of glycerokinase in the adipocytes (8). Finally, data in adult humans show that lipolysis in the adipose tissue is the major source of glycerol in blood (9). Thus, measurement of the Ra of glycerol in the circulation has been used to quantify the rate of lipolysis.

Diabetes in pregnancy exposes the fetus to a surfeit of metabolic nutrients and has been suggested to result in fetal and neonatal macrosomia (10). In spite of rigid control of maternal metabolism during pregnancy, fetal macrosomia and alterations in the concentration of circulating substrates persist in the neonate (10). Specifically, IDM have been shown to have lower concentrations of FFA in the immediate neonatal period, even though the plasma glucose and glycerol concentrations are similar to those in normal infants (3, 11). It has been hypothesized that the discrepancy between the plasma concentration of FFA and glycerol in the IDM may be related to increased reesterification of fatty acids in the adipocytes (3). In contrast, the SGA infant, as a result of intrauterine malnutrition, is born with decreased hepatic glycogen and adipose tissue triglyceride stores (12–14). These infants have lower respiratory RQ compared with the normal infants (5). In neither the IDM nor SGA infants have the quantitative contribution of fat to oxidative metabolism or the quantitative measurement of lipolysis been done.

Limited information is available regarding the quantitative role of glycerol as glucogenic precursor in the newborn infant (15). Such a role for glycerol may be particularly important in the presence of hypoglycemia, such as in IDM and SGA infants.

Abbreviations

AGA, appropriate for gestational age
SGA, small for gestational age
IDM, infant of diabetic mother

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Correspondence: Satish Kalhan, M.D., Division of Neonatology, Rainbow Babies and Children's Hospital, 2101 Adelbert Road, Cleveland, OH 44106.

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Using stable tracer-labeled glycerol and glucose in combination with respiratory calorimetry, the present study quantified the rates of glycerol turnover and its contribution to glucose and to expired CO₂ in the newborn infants of normal and diabetic mothers as well as in SGA infants. In addition, from the measurements of lipolysis and fat oxidation, estimates of triglyceride fatty acid cycling were obtained in these three groups.

MATERIALS AND METHODS

Subjects. Healthy newborns less than 48 h old were recruited from the normal newborn nursery. Twelve AGA infants, eight SGA infants, and five IDM were studied. Birth weight percentiles were derived from the Colorado intrauterine growth chart, and SGA was defined as birth weight less than the 10th percentile for gestational age. Clinical characteristics of the newborns are shown in Table 1. As expected at similar gestational age, SGA newborns weighed significantly less ($p < 0.001$) and IDM were significantly heavier ($p < 0.005$) as compared with the AGA newborns. During the first 24 h after birth, the SGA newborns and IDM had their blood glucose monitored by chemical strips (Dextrostix; Ames Laboratories, Elkhart, IN) every hour for the first 4 h and thereafter before every 3-h feeding. Two AGA infants and one IDM were studied before their first feed; two AGA infants were fed maternal breast milk, and all the remaining infants were fed a commercial formula (Enfamil). None of the newborns studied developed symptoms of hypoglycemia, received glucose or parenteral fluid, or were on any medication, including antibiotics.

The metabolism of women with diabetes was regulated by multiple injections of insulin and by diet throughout pregnancy. Four mothers were treated with insulin; one had gestational diabetes and was managed by dietary regulation. Their average HbA_{1c} concentration was $7.6 \pm 1.6\%$ (mean \pm SD) during the 3rd trimester (normal for our laboratory is $<6.5\%$).

The SGA infants were morphologically normal, had no evidence of intrauterine infection, and were born to healthy, normal women who had received no medication.

The study protocol was approved by the Institutional Committee on Investigation in Humans. After explanation of the study procedures, informed consent was obtained from the parents. The investigators were not responsible for the clinical care of the mother or the infant.

Study protocol. The studies were performed in the newborn metabolism study room in the Perinatal Clinical Research Center, where the ambient temperature was controlled at 28°C and humidity at 40%. The newborns had received their last feeding at least 3 h (range 2.75–7 h) before the start of tracer infusion, which lasted 5 h (Table 1). The infants were placed under the radiant warmer and thermoneutral environment was maintained throughout the study period. Two i.v. access lines were secured in the veins of opposite extremities using no. 23 scalp vein needles, for tracer infusion and for blood sampling. [¹³C] glycerol (71 atom % ¹³C; Merck Sharpe & Dohme Canada

Limited, Montreal, Canada) tracer solution was infused at a rate of $0.33 \pm 0.03 \mu\text{mol/kg}\cdot\text{min}$ using an occlusive pump (model 903; Extracorporeal Medical Specialties, Inc., King of Prussia, PA). Six AGA and six SGA newborns and two IDM also received a simultaneous infusion of [6,6-²H₂]glucose (98 atom % ²H; Merck, Sharpe & Dohme) tracer at a constant rate. No priming dose of the glycerol or glucose tracer was injected. Both tracers were infused for a period of 5 h. Plasma glucose concentration was monitored throughout the study to ensure that no infant became hypoglycemic.

The sampling site was kept patent by infusing normal saline at 5 mL/h. Heparin was not used for this purpose. Blood samples were obtained in heparinized syringes at 30-min intervals throughout the study, the sampling was less frequent in the SGA newborns. The total amount of blood drawn, in each infant, did not exceed 5% of the estimated blood volume (85 mL/kg). Blood samples, on ice, were taken immediately to the laboratory, where 100 μL of whole blood were used for evolution of CO₂ in vacuum; plasma from the rest of the sample was stored at -20°C . Indirect respiratory calorimetry was done at 30-min intervals (16). An open circuit flow-through system was used. A plastic hood was placed over the baby's head and a suction pump was used to draw air through the hood. The air exiting the hood was passed through a mixing chamber and the concentration of O₂ and CO₂ was measured using a medical gas analyzer (MGA-1100; Perkin-Elmer, Pamona, CA). The flow of air through the system was measured and maintained by a servo-control flow regulator (Brooks Instrument, Hatfield, PA). The accuracy and reproducibility of the system was validated by combustion of absolute ethyl alcohol in the system, which gave results within 3–5% of expected values. At the end of the study, infusate solution was checked for sterility and an aliquot saved for later analysis. Actual tracer infusion rate was determined by time-calibrating the infusion pump using the same infusion tubings and needles. The isotopic enrichment and purity of the tracer was confirmed by GC-MS analysis.

Sample analysis. CO₂ was evolved from the whole blood in vacuum by treating with perchloric acid and was collected by cryodistillation; its ¹³C:¹²C ratio was measured on a magnetic deflection double collector isotope ratio mass spectrometer (Nuclide Corp., State College, PA) (16). We have previously validated that the ¹³C enrichment in blood bicarbonate during [¹³C] glucose infusion studies is similar to ¹³C enrichment of breath CO₂ (16).

Mass-spectrometric analysis of glycerol and glucose. Two hundred μL of plasma were deproteinized with equal volumes of 0.32 M barium hydroxide and 0.32 M zinc sulphate. Preparatory purification was done by ion exchange chromatography (17). Acetate derivatives of glycerol and glucose were prepared by adding 10 μL of pyridine and 20 μL of acetic anhydride to the dried eluted samples. The reaction mixture was incubated at room temperature for 1 h. Derivatized samples were analyzed on the Hewlett-Packard GC-MS system (model 5985A; Hewlett-Packard Co., Palo Alto, CA). Samples were injected into a glass column (length 1.8 m, inner diameter 3.2 mm; Supelco, Inc.,

Table 1. Clinical characteristics of study subjects

	Weight (kg)	Gestational age (wk)	Apgar scores (5 min)	Age at study (h)	Duration of fast (h)*
AGA ($n = 12$)	$3.16 \pm 0.37^\dagger$	39.7 ± 1.2	8–9	20.8 ± 15.9 (2.8–45.5)	5.0 ± 1.3
IDM ($n = 5$)	$3.88 \pm 0.63^\ddagger$	39.2 ± 1.1	9	19.4 ± 15.2 (2.0–43.0)	4.4 ± 1.6
SGA ($n = 8$)	$2.12 \pm 0.37^\S$	38.5 ± 1.5	8–9	26.7 ± 14.6 (9.5–45.5)	4.2 ± 1.3

* Duration of fast before the start of tracer infusion, which lasted 5 h.

† Mean \pm SD.

‡ $p < 0.005$ when compared with AGA.

§ $p < 0.001$ when compared with AGA.

Bellefonte, PA) packed with 3% OV-17 mesh 100/120 (Alltech Associates, Inc., Applied Science Labs, Deerfield, IL). Helium was used as a carrier gas at a flow rate of 25 mL/min. The injection temperature was 220°C, column temperature was 200°C, and the GC-MS interphase temperature was 250°C. Glycerol triacetate had a retention time of 3–4 min. Selected ion monitoring software was used to monitor m/z 145 (m) and m/z 146 ($m + 1$), representing unlabeled and ^{13}C -labeled glycerol, respectively. After the elution of glycerol, the column temperature was increased to 240°C to elute glucose pentacetate, which had a retention time of 8–9 min. M/z 115 and m/z 116, representing carbon 2, 3, and 4 of unlabeled and labeled glucose, respectively, were monitored. Monitoring of larger ions of glucose, *e.g.* m/z 200 and m/z 201, showed that glucose had a significant contribution from recycled mono-deuterated glucose in the $[6,6\text{-}^2\text{H}_2]$ glucose infusion studies (18). Deuterium enrichment of glucose in the plasma was determined as described previously (17).

Plasma and infusate glycerol concentrations and plasma betahydroxybutyrate were measured by fluorometric modification of enzymatic micromethods (16, 19). Glucose was measured by coupled hexokinase and glucose-6-phosphate dehydrogenase method on a centrifugal analyzer (Baker Instruments, Allentown, PA), and FFA was measured by calorimetric micromethod. C-peptide and total and free insulin concentrations were measured by RIA (20). The lowest detectable levels by these methods were C-peptide 0.15 $\mu\text{g/L}$, total insulin 6 mU/L, and free insulin 4 mU/L.

Calculations. Glycerol and glucose turnover rates were calculated using the equation $R_a = [(100/\text{Ep}) - 1] \times I$, as described previously (21), where R_a = rate of appearance of substrate in the plasma ($\mu\text{mol/kg}\cdot\text{min}$), Ep = steady state isotopic enrichment of the substrate in the plasma, and I = infusion rate of the isotopic tracer ($\mu\text{mol/kg}\cdot\text{min}$).

$\dot{V}\text{O}_2$ (mL/kg \cdot min) and $\dot{V}\text{CO}_2$ (mL/kg \cdot min) were corrected by Haldane transformation and expressed at standard temperature and pressure. RQ was calculated as the ratio of $\dot{V}\text{CO}_2$ to $\dot{V}\text{O}_2$. Nonprotein RQ was calculated based upon an 8% contribution of protein to total caloric expenditure in fasted newborns (22). The precursor production relationships were calculated as follows: Fraction of glycerol to glucose ($\text{F}_{\text{glycerol} \rightarrow \text{glucose}}$) = $(\text{Glucose } R_a \times ^{13}\text{C enrichment glucose} \times 2) / (\text{I}_{\text{glycerol}} \times 0.71)$, where Glucose R_a is the rate of appearance of glucose and the ^{13}C enrichment of plasma glucose represents carbons 2, 3, and 4 of glucose. The product of Glucose R_a and ^{13}C enrichment glucose was multiplied by 2, assuming a symmetrical distribution of C-2 of glycerol on carbon 2 and 5 of glucose. $\text{I}_{\text{glycerol}}$ was the infusion rate of tracer glycerol, and 0.71 was the ^{13}C enrichment of infused glycerol tracer.

Fraction of glucose from glycerol = $(\text{Glucose } R_a \times 2) / (\text{Glycerol } R_a \times \text{fraction of glycerol to glucose})$.

Fraction of glycerol to CO_2 ($\text{F}_{\text{glycerol} \rightarrow \text{CO}_2}$) = $(\dot{V}\text{CO}_2 \times ^{13}\text{C enrichment of CO}_2) / (\text{I}_{\text{glycerol}} \times 0.71)$.

Fraction of CO_2 from glycerol ($\text{F}_{\text{CO}_2 \leftarrow \text{glycerol}}$) = $\dot{V}\text{CO}_2 / (\text{Glycerol } R_a \times \text{Fraction of glycerol to CO}_2 \times 3)$.

The triglyceride-fatty acid cycling was quantified as described by Elia *et al.* (23). The amount of energy mobilized from adipose tissue was calculated from the release of glycerol (R_a), assuming that 1 mol of glycerol is released from 1 mol of triglyceride (dioleoyl-palmityl-triglyceride = 8094 kcal/mol). Quantitative estimates of fat oxidation were obtained from respiratory calorimetry measurements. The difference between energy mobilized and fat oxidized represents the amount of triglycerides recycled back to fat stores (23, 24).

Statistical analysis. IDM and SGA groups were compared with the AGA group using two-tailed *t* test. Data within the group were compared using paired *t* test. Differences were considered significant at $p < 0.05$.

RESULTS

Substrate concentrations (Table 2). At the start of the study, the plasma glucose concentration was significantly lower in the IDM ($p < 0.05$) and the SGA infants ($p < 0.005$) as compared with the AGA infants. As a result of continued fasting, there was a small decrease in glucose concentration in the AGA infants by the end of the tracer infusion period (Fig. 1). No decrease in glucose concentration was observed in IDM or SGA infants. However, after 8 h of fasting, plasma glucose was still significantly lower in SGA as compared with AGA infants ($p < 0.01$).

There was no difference in the plasma glycerol (Fig. 2) and FFA concentrations in the three groups, neither at the beginning nor at the end of the study. The concentration of both glycerol and FFA was higher at time 0 than at 60 min, probably representing the stress of needle puncture and other manipulations. The betahydroxybutyrate concentration was lower, although not statistically significant, in the IDM and SGA infants ($p = 0.06$) as compared with AGA infants. There was a continuous increase in betahydroxybutyrate concentration throughout the study so that by 300 min betahydroxybutyrate concentrations had significantly increased from the basal levels in both AGA ($p < 0.001$) and SGA ($p < 0.01$) infants.

The mean plasma C-peptide concentration in IDM infants ($0.71 \pm 0.14 \mu\text{g/L}$) was significantly greater compared with that in AGA infants ($0.30 \pm 0.14 \mu\text{g/L}$, $p < 0.01$). Although total insulin was higher in IDM as compared with AGA infants, the free insulin levels were similar in the two groups (AGA $4.4 \pm 0.79 \text{ mU/L}$, IDM $5.3 \pm 0.05 \text{ mU/L}$). Plasma was insufficient to measure insulin in SGA infants.

Respiratory calorimetry. $\dot{V}\text{O}_2$, $\dot{V}\text{CO}_2$, and RQ were measured in a quiet state. Two to four recordings of 15–20 min each over 1–2 h were averaged in each newborn. As shown in Figure 3, the $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$ were similar in the three groups. RQ was also not significantly different in the IDM and SGA infants when compared with AGA infants.

Glycerol kinetics in newborn. The ^{13}C enrichment of plasma glycerol, glucose [m/z 116/115, representing C2, 3, and 4], and expired CO_2 for the AGA infants are displayed in Figure 4. As shown, a steady state was achieved during the last 3 h of the study for glycerol and during the last 2 h for glucose. The ^{13}C enrichment of the plasma bicarbonate approached plateau by 4½–5 h. Because plateau enrichment of CO_2 was not observed in all instances, it may have resulted in underestimation, probably small, of the contribution of glycerol carbon to CO_2 .

The R_a of glycerol, calculated by tracer dilution during steady state, was similar in the AGA infants and IDM—approximately $9.5 \mu\text{mol/kg}\cdot\text{min}$ (Table 3). Glycerol R_a was significantly higher ($p < 0.05$) in the SGA infants. Glucose R_a was quantified in the six SGA and six AGA infants. The glucose R_a was more variable in SGA infants; however, mean glucose turnover rate was not significantly different as compared with that in AGA infants. The rate of glucose turnover in two IDM was 28 and 26 $\mu\text{mol/kg}\cdot\text{min}$. No correlation between glycerol flux and plasma glycerol concentration was observed in any group or in the entire infant population.

Fate of glycerol carbon in human newborn. The contribution of glycerol to glucose and to expired CO_2 was calculated by examining the precursor-product relation. As shown (Table 3), the major component (over 81%) of glycerol flux was converted to glucose. The glucogenic contribution of glycerol ($\text{F}_{\text{glucose from glycerol}}$) was determined to be 20% in AGA infants, SGA infants, and the two IDM.

During the last 30 min of the study, approximately 35% of the glycerol carbon was recovered in the blood bicarbonate pool, and 5% of the CO_2 produced could be accounted for by glycerol oxidation (Table 4). The fraction of CO_2 derived from glycerol and the fraction of glycerol to CO_2 was similar in the three groups.

There was a significant positive correlation between glycerol

Table 2. Plasma concentration of circulating substrates during study*

	Glucose (mM/L)		Glycerol (μ mol/L)		FFA (mmol/L)		B(OH) butyrate (mmol/L)	
	0 h	5 h	0 h	5 h	0 h	5 h	0 h	5 h
AGA	3.23 \pm 0.80	2.99 \pm 0.24	231 \pm 72	159 \pm 40	1.07 \pm 0.58	0.84 \pm 0.48	0.40 \pm 0.33	0.59 \pm 0.39†
IDM	2.41 \pm 0.93‡	2.74 \pm 0.54	248 \pm 67	160 \pm 39	1.11 \pm 0.42	0.79 \pm 0.23	0.22 \pm 0.10	0.33 \pm 0.21
SGA	2.13 \pm 0.67§	2.39 \pm 0.51	178 \pm 77	138 \pm 56	1.20 \pm 0.83	0.96 \pm 0.49	0.19 \pm 0.12	0.35 \pm 0.09¶

* The data at 5 h are the mean of observation during the 4th and 5th h of tracer infusion.

† Paired *t* test with time 0, $p < 0.01$.

‡ $p < 0.05$ compared with AGA infants.

§ $p < 0.005$ compared with AGA infants.

|| $p < 0.01$ compared with AGA infants.

¶ Paired *t* test with time 0, $p < 0.01$.

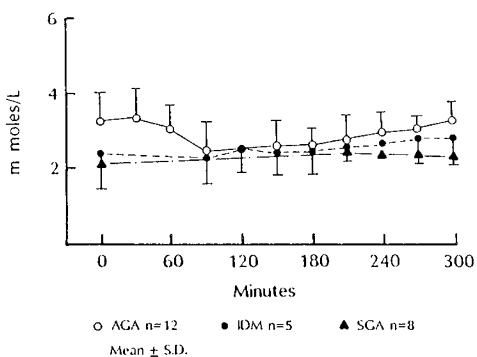


Fig. 1. Plasma glucose concentration in the newborn infants during the study (mean \pm SD). The infants were fasted for at least 3 h before the study (0 min) and remained fasted throughout the 300 min of the study period.

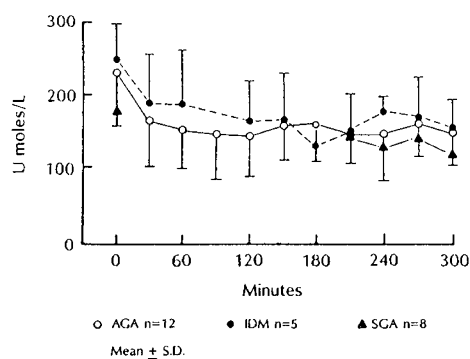


Fig. 2. Plasma glycerol concentration in newborn infants during the study (mean \pm SD). The infants were fasted for at least 3 h before the study (0 min) and remained fasted throughout the 300 min of the study period.

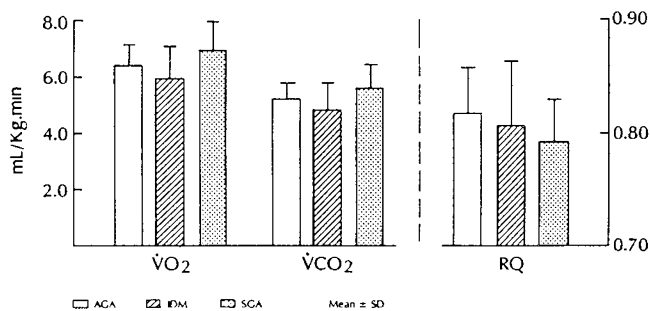


Fig. 3. Rate of $\dot{V}O_2$, rate of $\dot{V}CO_2$, and RQ.

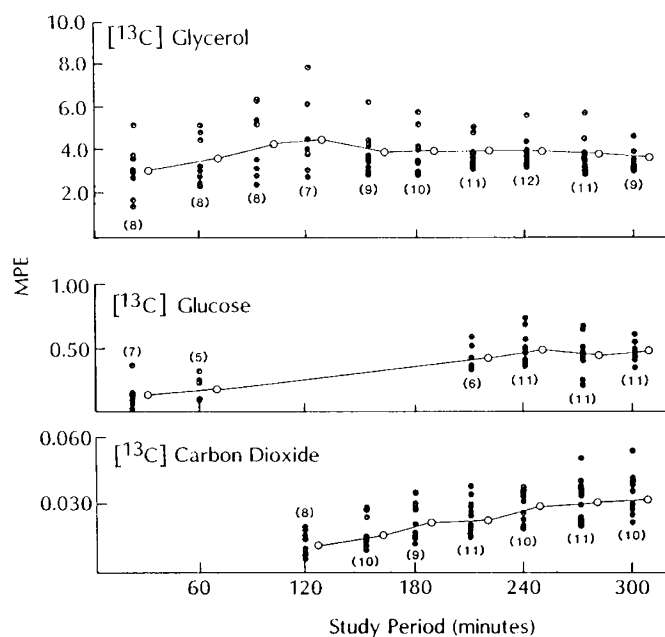


Fig. 4. Changes in ^{13}C enrichment of glycerol, glucose, and CO_2 (bicarbonate) throughout the study. $[2-^{13}C]$ glycerol was infused at a constant rate in 12 AGA newborns. MPE, moles % excess (the natural background enrichment has been subtracted from the data reported).

flux and the fraction of CO_2 from glycerol in AGA infants and IDM (Figs. 5 and 6).

$$\text{AGA } y = -0.821 + 0.53x; p = 0.001; r = 0.84$$

$$\text{IDM } y = 0.127 + 0.49x; p = 0.02; r = 0.93$$

Triglyceride-fatty acid cycle. The triglyceride energy mobilized was calculated from the rate of glycerol release. As shown in Table 5, the energy released from the hydrolysis of triglyceride was increased in the SGA infants. However, only 22 to 24% of energy released was oxidized to CO_2 , with the majority (76–78%) being recycled back to the adipose tissue. This estimation represents the total triglyceride-fatty acid cycle and does not distinguish between extra- and intracellular compartments. The energy cost (kcal/kg·d) of triglyceride recycling was 1.52 ± 0.46 in AGA infants, 1.54 ± 0.42 in IDM, and 2.08 ± 0.86 in SGA infants, representing 3.5, 3.9, and 4.4% of the resting energy consumption, respectively.

DISCUSSION

In the present study, using isotopic tracer methods and respiratory calorimetry, we have observed that 1) lipolysis, as measured by glycerol release, is active in the newborn infant; 2) glycerol is a significant glucogenic precursor in the newborn

Table 3. Glycerol-glucose metabolism in newborn*

	Glycerol turnover ($\mu\text{mol}/\text{kg}\cdot\text{min}$)	Glucose turnover ($\mu\text{mol}/\text{kg}\cdot\text{min}$)	F Glycerol \rightarrow glucose	Q Glycerol \rightarrow glucose ($\mu\text{mol}/\text{kg}\cdot\text{min}$)	F Glucose \leftarrow glycerol	Q Glucose \leftarrow glycerol ($\mu\text{mol}/\text{kg}\cdot\text{min}$)
AGA	9.47 \pm 2.11 (n = 12)	21.09 \pm 1.71 (n = 6)	0.87 \pm 0.16 (n = 6)	8.69 \pm 2.60 (n = 6)	0.20 \pm 0.06 (n = 6)	4.34 \pm 1.30 (n = 6)
IDM	9.55 \pm 2.14 (n = 5)	28.3, 26.6 (n = 5)	0.9, 1.2 (n = 5)	11.41, 9.92 (n = 5)	0.20, 0.19 (n = 5)	5.71, 4.96 (n = 5)
SGA	12.15 \pm 3.87† (n = 8)	23.08 \pm 4.74 (n = 6)	0.82 \pm 0.25 (n = 6)	9.05 \pm 2.92 (n = 6)	0.20 \pm 0.06 (n = 6)	4.52 \pm 1.46 (n = 6)

* F, fraction of; Q, quantity of.

† $p < 0.05$ (0.03) as compared with AGA infants.

Table 4. Oxidative fate of glycerol carbon in human newborn*

	Glycerol Ra ($\mu\text{mol}/\text{kg}\cdot\text{min}$)	$\dot{V}\text{CO}_2$ ($\mu\text{mol}/\text{kg}\cdot\text{min}$)	F Glycerol \rightarrow CO ₂	F CO ₂ \leftarrow glycerol
AGA	9.47 \pm 2.11 (n = 12)	229 \pm 29 (n = 12)	0.33 \pm 0.05 (n = 11)	0.044 \pm 0.013 (n = 11)
IDM	9.55 \pm 2.14 (n = 5)	211 \pm 40 (n = 5)	0.36 \pm 0.08 (n = 5)	0.049 \pm 0.011 (n = 5)
SGA	12.15 \pm 3.87† (n = 8)	246 \pm 42 (n = 6)	0.36 \pm 0.08 (n = 6)	0.046 \pm 0.012 (n = 6)

* F, fraction of.

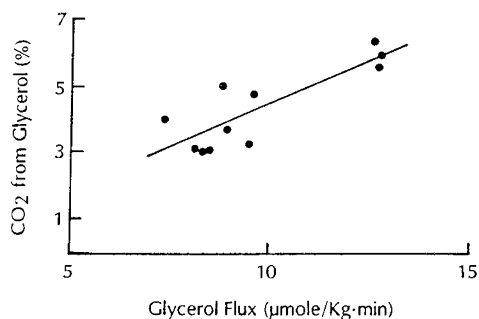
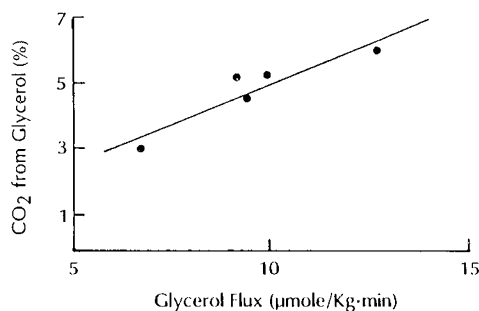
† $p < 0.03$ compared with AGA infants.Fig. 5. Relation between fraction of CO₂ from glycerol and glycerol flux in AGA infants. $y = -0.82 + 0.53x$; $r = 0.84$; $p = 0.001$.Fig. 6. Relation between fraction of CO₂ from glycerol and glycerol flux in IDM. $y = 0.127 + 0.49x$; $r = 0.93$; $p = 0.02$.

Table 5. Triglyceride-fatty cycle in newborn infants

	Triglyceride energy mobilized ($\text{kcal}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$)	Fatty acid oxidized ($\text{kcal}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$)	Recycled
Normal	110.4 \pm 24.6	24.8 \pm 6.4	0.76 \pm 0.07
IDM	111.3 \pm 24.9	24.9 \pm 10.4	0.77 \pm 0.10
SGA	141.6 \pm 45.1	30.4 \pm 6.3	0.78 \pm 0.08

infant, representing as much as 20% of glucose turnover; 3) 35% of glycerol carbon is converted to CO₂, representing 5% of CO₂ production, and glycerol oxidation to CO₂ is via its conversion to glucose; 4) possibly as a result of improved management of

maternal metabolism, glycerol metabolism in the IDM was noted to be normal; and 5) lipolysis was observed to be increased in SGA infants.

The following potential problems in the experimental methods and the expression of data, unique to the newborn population, need to be recognized: 1) We had chosen to infuse the tracer in the peripheral vein and obtain blood samples from the peripheral vein of the opposite extremity. Although venous-arterial infusion sampling mode, *i.e.* tracer infusion in a vein and sampling of arterial blood, may be the most appropriate method for quantifying glycerol kinetics (25–27), ethical considerations do not permit placement of arterial lines in healthy newborn infants. In any case, the error as a result of tracer dilution by the glycerol release from the hand in a blood sample obtained from the dorsum of the hand should be small when compared with the total glycerol turnover. Similarly, although some glycerol can be oxidized by the muscle, at least in the human adult (28), the contribution of the local muscle oxidation in relation to the total body glycerol oxidation is likely to be small, particularly in view of the observation that more than 80% of glycerol was converted to glucose. Therefore, the ¹³C enrichment of bicarbonate in the venous blood should have minimal contribution from local glycerol oxidation. Thus, one can assume that the reported measurements of glycerol metabolism are close approximations of the actual kinetics. 2) All the data have been expressed in relation to total body weight. Although the data on energy consumption and maybe glycerol kinetics could be better expressed in relation to lean body mass, calculation of the data based upon lean body mass is not likely to make any difference in the results. Specifically, the small but not significant differences among the three groups in $\dot{V}\text{O}_2$ measurements (Fig. 3) will actually disappear when the data are expressed in relation to lean body mass, without having any effect on RQ measurement. The higher glycerol turnover rates in the SGA infants are consistent with the lower RQ observed in this group.

Glycerol turnover in newborn infants. The rate of turnover of glycerol in healthy, normal AGA infants was 9.5 $\mu\text{mol}/\text{kg}\cdot\text{min}$. Bougneres *et al.* (15), using a similar isotopic tracer method and under similar experimental conditions in five infants, estimated the turnover rate of glycerol to be 4.4 $\mu\text{mol}/\text{kg}\cdot\text{min}$. The reason for these differences remains unclear and may be related to other factors such as prior glucose infusion and subtle clinical differences in the study populations. In addition, the total duration of the fast, from the last meal to the time of isotopic steady state measurements, in our study was 7 to 9 h, in contrast to the previous study, where it was maximally 6 h. All infants in the present study were healthy, full-term, AGA infants and were not receiving glucose or antibiotics for any clinical indications.

In lean, healthy adults, after an overnight fast, the rate of glycerol turnover has been reported to be 1.5–2.5 $\mu\text{mol}/\text{kg}\cdot\text{min}$ (23, 29–31). With prolonged fasting, an increase in lipolysis and glycerol turnover has been reported so that between 3 and 15 d of fasting the rate of glycerol flux ranged between 3.3 and 5.8 $\mu\text{mol}/\text{kg}\cdot\text{min}$ (30, 31). The rates of glycerol flux in the newborn infant in the present study were much higher than those observed in adults, even after prolonged fasting. Lipolysis has been considered to reflect the energy requirement of the lean body mass

(32). Even though the lean body mass of the newborn is less compared with that of the adult (40 versus 70%), the greater energy requirements of the neonate will result in early depletion of glycogen stores and an early recruitment of fat for energy and thus a higher flux rate of glycerol and fatty acids in the infants. The higher flux rates of these substrates are also reflected in higher circulating levels of FFA and glycerol. The mediators of these responses in the newborn may be the lower insulin levels and the relatively higher catecholamine levels during the immediate postnatal period of extrauterine adaptation. In addition, as shown by Marcus *et al.* (33), thyrotropin may have a major lipolytic role in this age group.

Glycerol-glucose relationship. The major metabolic fate of glycerol in man and animals is conversion to glucose. The key enzyme involved in the regulation of the utilization of glycerol, glycerokinase, is present in significant quantities only in liver, kidney, and small intestine. The major site of gluconeogenesis from glycerol is liver and kidney (8, 34). Vernon and Walker (35) have demonstrated a marked increase after birth in the ability of both the liver and kidney slices from the neonatal rat to convert glycerol into glucose plus glycogen. In addition, this increase was correlated with the increase in glycerol kinase activity. Similar observations of an increase in enzymes of glycerol metabolism in the liver after birth were reported by other investigators (36, 37). Quantitatively, in the 0- to 4-d-old newborn dogs, Hall *et al.* (38) reported that approximately 50% of glycerol carbon was converted to glucose, accounting for 14% of glucose carbon. This was in contrast to adult dogs, in which glycerol accounted for only 6% of glucose carbon released in circulation (38, 39). In lean and obese human adults after a short fast, 38 and 56%, respectively, of glycerol turnover is converted to glucose, representing 3 and 8%, respectively, of glucose released in blood (31). A prolonged fast resulted in a marked increase in both conversion of glycerol to glucose and in the contribution of glycerol to glucose turnover (31). In contrast, in the normal newborn infants in the present study, 87% of glycerol turnover was converted to glucose after 7–9 h of fasting, representing 20% of glucose turnover. These data are of similar magnitude as those seen in human adults after a prolonged fast (31) and demonstrate the speed by which a neonate can mimic the fasting state in adults, possibly because of its high energy demands in relation to available glycogen stores. Our data are similar to those reported by Bougneres *et al.* (15), who showed that 73% of glycerol turnover was converted to glucose in five full-term infants. However, they reported that a maximum of 7% of glucose turnover could possibly come from glycerol. The difference between their study and the data reported by us primarily lies in the differences in glucose turnover rates measured in the two studies. Again, these differences may be related to the duration of fasting and the clinical study populations.

Two methodologic problems need to be considered in relation to glycerol-glucose relationship. First, we have assumed an equal distribution of the tracer ^{13}C in carbon 2 and 5 of glucose. Studies in the rat have demonstrated an asymmetrical distribution (40, 41). Although no such data are available, particularly in the human newborn infants, our calculation of the data, assuming symmetrical distribution, would result in a small overestimation (~17%) of glycerol's contribution to glucose. Second, some of the tracer ^{13}C incorporated into glucose could recycle back to glucose after the metabolism of glucose to pyruvate, resulting in a higher ^{13}C enrichment of glucose (18). However, the recycled tracer carbon (^{13}C) will be significantly diluted. Furthermore, randomization of carbon during resynthesis of glucose and the ion species measured, representing carbon 2, 3, and 4 of glucose, will further decrease the possibility of this overestimation (18).

Oxidation of glycerol in neonates. In the newborn infant, about 33% of glycerol appearing in the circulation was oxidized to CO_2 ; however, it contributed less than 5% to overall VCO_2 and thus to energy metabolism. Almost all of the glycerol oxidized to CO_2 was via its first conversion to glucose. As in any precursor-

product relationship measured by tracer isotopes, the oxidation of glycerol could potentially be underestimated because of dilution of the tracer in the intermediate pools. Nevertheless, these data show that glycerol is a minor source of energy for the newborn. No other data regarding glycerol oxidation in the human newborn have been reported. The present estimate by tracer isotope methods is similar to those derived from the previously reported rates of glucose oxidation in the newborn (~50%) and the reported rate of conversion of glycerol to glucose (~70–80%) (16). Similar to the data in infants, in the human adult only 2–5% of CO_2 was derived from glycerol after a short fast and only a small increase was seen when fasting was prolonged (39). It is significant to note that after a prolonged fast in these adult subjects there was a marked increase in glycerol turnover. These latter data are in contrast to those from infants, in whom the fraction of CO_2 derived from glycerol was significantly correlated with the glycerol flux (Figs. 5 and 6). Again, these differences in the adult and the newborn are probably related to the differences in the rates of energy consumption in the two groups. Further, the increased oxidation of glycerol with increased glycerol flux may result in sparing of other metabolizable fuels, particularly proteins.

Glycerol metabolism in IDM. Our study did not demonstrate any significant effect of maternal diabetes upon neonatal glycerol and fat metabolism. Even though the C-peptide levels were significantly elevated in the IDM, their free insulin concentration was similar to that in normal infants. Furthermore, the plasma concentrations of glucose, FFA, and glycerol were comparable in the two groups. These data suggest that, possibly as a result of rigorous regulation of maternal metabolism during pregnancy, the glycerol and energy metabolism in the IDM was normalized. Our data do not present evidence for increased reesterification of FFA in the adipose tissue of IDM as hypothesized previously (3).

SGA infants. As anticipated, the SGA infants, who are born with relatively lower glycogen stores, had higher rates of lipolysis and a slightly increased ($p = 0.06$) contribution of fat to energy metabolism. Their plasma glucose concentration was lower than that in AGA infants and remained low throughout the study. The parameters of glycerol metabolism were similar in the SGA and AGA infants. These infants, however, were not severely SGA; thus, marginal malnutrition appears to cause minor changes in fat and energy metabolism.

Triglyceride-fatty acid cycle in newborn infants. Quantitative estimates of the triglyceride-fatty acid substrate cycle have been made *in vivo* in adult man and animals as well as *in vitro* in isolated adipose tissue (23, 24, 42–44). These data show that only a small fraction, 20–30%, of the fatty acid released from the adipose tissue is oxidized and that the majority of the released fatty acids are recycled back to adipose tissue and resynthesized into triglycerides. The differences in estimates observed in various studies probably reflect the differences in experimental techniques used and the metabolic state of the individuals (23, 42, 43). Nevertheless, the data in adult humans and animals suggest that the triglyceride-fatty acid cycle is regulated by catecholamines and insulin and that it plays an important role in energy metabolism in humans (24). Specifically, it has been suggested that changes in substrate cycling contribute to the increased thermogenesis after severe burns in humans (45), to the increased metabolic rate of cachectic patients with esophageal cancer (46), and to the amplification of substrate flux and responsiveness during exercise (43). Finally, triglyceride-fatty acid cycle has been observed to be markedly increased in states of substrate privation such as short-term fasting in man (30). The present data are the first estimate of triglyceride-fatty acid cycling in newborn infants. Interestingly, these data show that although the magnitude of lipolysis expressed per unit body weight is markedly increased in newborn infants compared with adults, the fraction of fatty acids released that was recycled back to triglycerides was of similar magnitude (~75%) as was seen in

adults. Furthermore, no effect of maternal diabetes on triglyceride-fatty acid cycling in the infant was observed. This may be the result of good management of maternal diabetes, because, unlike the previous observations, the free insulin levels in the IDM were similar to those in normal newborn infants. In addition, the increased fatty acid oxidation observed in SGA infants was associated with increased triglyceride mobilization as well as recycling. It is significant to note that the fraction of triglyceride energy recycled was similar in the three groups (76%) and that energy cost of the recycling represented similar fractions of the resting energy consumption in the three groups. Thus, as suggested by Newsholme and Crabtree (47), these high rates of substrate cycling in the newborn infants may provide increased sensitivity or the ability to amplify metabolic control systems when required.

In summary, our data show that lipolysis is active in the immediate neonatal period in normal infants, SGA infants, and IDM. Lipolysis and the contribution of fat to oxidative metabolism was increased in SGA infants. The major metabolic fate of glycerol in the human newborn was shown to be conversion to glucose and to energy substrate via its conversion to glucose. Our data show that triglyceride-fatty acid cycling is markedly increased in the newborn compared with adults. Finally, maternal diabetes and intrauterine growth retardation did not have any effect on these parameters.

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