Lipid Transport in the Human Newborn

PALMITATE AND GLYCEROL TURNOVER AND THE CONTRIBUTION OF GLYCEROL TO NEONATAL HEPATIC GLUCOSE OUTPUT

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ABSTRACT Free fatty acid (FFA) transport was measured in 11 and glycerol turnover in 5 newborns with continuous tracer infusion of [1-^{13}C]palmitate or [2-^{13}C]glycerol, respectively. In addition, simultaneous determination of glucose production in the latter group with [6,6-^{2}H$_2$]glucose tracer and measurement of the appearance rate of [^{13}C]glucose derived from [^{13}C]glycerol allowed calculation of gluconeogenesis from glycerol.

The average FFA inflow rate was 11.5±1.7 μmol kg^{-1}min^{-1}, 2.5–4.5 h after the last feeding, and 16.7±2.8 μmol kg^{-1}min^{-1}, 5–12 h after the last meal. These rates are comparable to those found in adults only after 8–16 h and ~72 h of fasting, respectively. FFA inflow in the newborn was directly correlated with time of fasting, plasma FFA level, and plasma glycerol level. Palmitate clearance and fractional removal were inversely related to palmitate level.

Glycerol flux averaged 4.4±0.5 μmol kg^{-1}min^{-1}, a value three- to fourfold that of the postabsorptive adult. Approximately 75% of transported glycerol was converted to glucose and represented 5.0±0.6% of hepatic glucose production. Furthermore, there was a direct relationship between glycerol turnover and the fraction of glucose coming from glycerol.

Despite the absolutely elevated neonatal FFA and glycerol transport rates, these were quantitatively similar to values found in adults with comparable elevated substrate levels. Furthermore, other similarities with the adult in the relationships between inflow transport and substrate values, and between transport and fractional removal suggest that the regulatory aspects of lipid transport in man are already well developed by the first day of life.

INTRODUCTION

The transition from intrauterine to extrauterine life is, of necessity, accompanied by major alterations in fuel homeostasis, largely the result of abrupt termination of the major fetal fuel, glucose. Although the human neonate is capable of producing glucose at a rate of ~6 mg kg^{-1}min^{-1} (1), sufficient to meet cerebral energy needs, daily glucose production alone can barely satisfy the minimal metabolic rate of the term infant on the first day of life (2) and is far short of meeting the total energy requirements of the active, growing neonate (2, 3). Thus, because exogenous caloric intake is generally inadequate during early postnatal life, there is an absolute need for other oxidizable endogenous substrates to meet the infant's energy demands. As in the adult, mobilized fatty acids are the primary energy source. Immediately after birth, there is a dramatic rise in circulating free fatty acids (FFA)[1] (4–7) and a simultaneous fall in respiratory quotient from above 0.8 to near 0.7 (8–10). In addition, compared with other species, the newborn has a dramatic accumulation of adipose tissue near the end of gestation (11), and has large fat stores approximating 10–15% of body weight at birth (11–13). In vitro, this adipose tissue is capable of releasing large amounts of FFA(14). Furthermore, because lipolysis of adipose tissue triglyceride also releases glycerol into the circulation, accelerated lipid mobilization in the neonatal period might serve the important additional function

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[1] *Abbreviations used in this paper:* FFA, free fatty acid; R$_a$, rate of appearance; R$_c$, clearance rate; R$_d$, rate of disappearance.

of supplying substantial gluconeogenic substrate in the form of glycerol.

Nevertheless, there are no quantitative measurements of FFA and glycerol transport or of glycerol's contribution to hepatic glucose output in the human newborn. Largely, this deficiency has been due to the unavailability of ethically acceptable means for obtaining these data in neonates. Recently developed gas chromatography-mass spectrometry (GCMS) approaches using nonradioactive, carbon-13-labeled substrate tracers have allowed us to make these measurements in the human neonate.

METHODS

Patients. FFA turnover was measured in 11 infants (group A), and glycerol transport and gluconeogenesis from glycerol was determined in 5 newborns (group B) whose clinical characteristics are presented in Table I. 10 were boys and 6 were girls. 10 were term infants, all of whom were appropriately-sized-for-gestational age. 6 were born prematurely and 2 of these infants (numbers 7 and 10) were small-for-gestational age. Because there were no discernable differences in the substrate and kinetic results related to gestational age, the subjects are subsequently discussed without reference to gestational age. Each of the subjects was

born after an uncomplicated pregnancy, but infant number 8 had a low initial Apgar score responding to routine resuscitation, 9 mothers had premature rupture of membranes, and 1 mother of fraternal twins (numbers 14 and 15) had a temperature of 38°C during labor. Thus, 11 of the infants studied were receiving intravenous antibiotic therapy, but in none was infection confirmed with culture evidence.

At the time of study, all but one of the infants was asymptomatic and resting comfortably in an isolette. Infant number 9 had mild respiratory distress syndrome and was studied in an isolette with a 40% inspired O_2 concentration. During the study period each infant was covered with a blanket to avoid hypothermia and rectal temperatures taken at the beginning and end of each study did not fall below 36.5°C.

Medical care of each newborn studied was under control of the child's personal physician who designated the infant's feeding schedule. Thus, while there is a broad postprandial study range, no infant was specifically fasted for the purpose of the tracer experiment. Subjects 5 and 9 had received no oral feedings before study. These two infants, and the remaining 9 neonates treated with intravenous antibiotics, were receiving dextrose intravenously at a "keep open" rate of <1 mg dextrose $kg^{-1}min^{-1}$ except for newborn number 9 whose dextrose infusion rate was 5.8 mg $kg^{-1}min^{-1}$. In each instance, the intravenous solution was switched to 0.45% saline a miminum of 1.5 h (and generally 3 h) before starting the tracer experiment.

Materials. [1-^{13}C]palmitic acid (90 atom % 1-^{13}C), [5,5,-6,6-^{2}H$_4$]palmitic acid (96 atom % ^{2}H$_4$), [2-^{13}C]glycerol (94

TABLE I
Clinical Characteristics of the Patients Studied

Subject	Sex	Gesta-tional age	Weight*	Weight	Postnatal age*	Time* since last feeding	Intrapartum history	Apgar scores	Condition at time of study
		wk	kg	percentile	h	h		1 min/ 5 min	
FFA study (group A)									
1	M	42	4.20	90	24	4	PRM‡	8/9	well
2	M	40	2.91	15	23	5	PRM; C-Sect.‖	7/9	well
3	M	40	3.53	70	15	4.5	PRM	9/9	well
4	M	38	2.60	10	18	2	PRM	8/9	well
5	M	39	2.93	15	10	10	PRM	9/10	well
6	M	37	2.67	25	46	2.5	PRM	6/8	well
7	F	36	2.00	3	20	2	unremarkable	8/9	well
8	M	34	2.20	85	20	2	unremarkable	4/7	well
9	F	35	2.03	10	20	1.5	PRM	2/8	RDS,§ 40% O_2
10	M	34	1.56	1	16	2	PRM	6/8	well
11	M	40	3.27	35	16	3.5	unremarkable	8/9	well
Glycerol study (group B)									
12	M	40	3.06	20	24	2.5	unremarkable	7/9	well
13	F	36	2.56	60	20	2	PRM	9/9	well
14	F	39	2.70	10	20	4	maternal fever	8/9	well
15	F	39	2.90	15	22	4	maternal fever	8/9	well
16	F	40	3.00	20	23	4	unremarkable	8/9	well

* At onset of tracer infusion study.
‡ PRM = premature rupture of membranes.
§ RDS = respiratory distress syndrome.
‖ C-Sect = Caesarean section.

atom % 2-^{13}C], and D-[6,6-^{2}H$_2$]glucose (97 atom % ^{2}H$_2$) were purchased from Merck, Sharp and Dohme Canada, LTD., Montreal, Quebec, Canada. Chemical and isotopic purity were confirmed by conventional gas chromatographic and mass spectrometric analyses. Each preparation was pyrogen free by standard rabbit body temperature measurements after intravenous administration according to federal guidelines in a licensed commercial laboratory. [6,6-^{2}H$_2$]glucose and [2-^{13}C]glycerol were dissolved in 0.5 N saline and prepared for human use as described (1). [1-^{13}C]palmitic acid was bound to fatty acid-free human albumin for intravenous administration as described (15).

Procedural methods. Infants were studied after obtaining written parental consent according to protocols approved by the Washington University Committee on Human Experimentation. In 11 of the infants, the peripheral intravenous "scalp vein" needle already in place for antibiotic administration was used for tracer infusion. In the remaining 5 infants a 25-gauge scalp vein needle was inserted into a peripheral vein, the infant was returned to the isolette, covered with a blanket, and allowed to rest and stabilize body temperature for at least 1 h before the onset of tracer infusion. At time zero of study, "base-line" samples were collected (see below) and the continuous infusion of labeled substrate was begun using a calibrated Harvard syringe pump (Har-

vard Apparatus Co., Inc., S. Natick, MA) at the rate of 0.205±0.021 (SE) μmol kg^{-1}min^{-1} [1-^{13}C]palmitic acid (group A); 0.242±0.007 μmol kg^{-1}min^{-1} [6,6-^{2}H$_2$]glucose and 0.311±0.011 μmol kg^{-1}min^{-1} [2-^{13}C]glycerol (group B) except for the first infant in the glycerol study (number 12) who received [2-^{13}C]glycerol at the rate of 0.429 μmol kg^{-1}min^{-1}. These rates were maintained for 120 min in the former and 135 min in the latter group.

Before the start of the tracer infusion and every 15 min from 60 to 120 or 135 min, 400 μl arteriolized "heel stick" capillary blood samples were collected into iced centrifuge tubes, and the plasma was immediately separated by high-speed centrifugation. For the FFA study (group A) albumin-bound [^{2}H$_4$]palmitic acid internal standard (see below) was added to a 100-μl aliquot of the subject's plasma, which was then placed immediately into 5 ml of Dole's mixture (isopropanol/heptane/H$_2$SO$_4$, 40:10:1), and the fatty acids were extracted by vigorous shaking. Plasma proteins were precipitated from an additional 50 μl of plasma by addition to an equal volume of cold 3 M perchloric acid for subsequent substrate measurement (see below). In certain instances (Table II) inadequate sample size limited substrate measurement. For the glycerol study (group B), 50 μl of plasma was prepared for substrate determination as above. The remaining 100–150 μl of plasma was added to 0.5 ml of 0.3 N bar-

TABLE II
Substrate Values during the Tracer Infusion Study*

Subject	Plasma glucose	Plasma palmitate	Plasma FFA	Plasma glycerol	Plasma β-hydroxybutyrate
	mg/dl	*μM*	*μM*	*μM*	*μM*
FFA study (group A)					
1	54±1	559±28	1,339±41	327±15	167±6
2	49±5	415±14	1,026±13	147±8	89±6
3	63±3	257±55	851±160	243±17	116±9
4	91±5	81±4	230±8	360	40±5
5	66±6	462±15	1,109±35	—	281
6	87±2	185±6	637±16	148	74±2
7	42±1	100±11	272±29	176±23	34±3
8	58±2	131±21	422±70	104	54±5
9	50±12	522±50	940±91	—	259
10	56±3	125±12	420±39	155	38±1
11	80±2	431±17	956±48	270±6	155±7
Mean	63	297	746	214	119
SE	±4.9	55	112	27	26
Glycerol study (group B)					
12	57±2	99	289	68±3	52±3
13	62±2	81	230	46±2	39±3
14	62±1	173	665	68±3	66±4
15	70±3	302	1,165	74±2	53±3
16	53±1	263	1,039	137±1	74±3
Mean	61	184	677	79	57
SE	3	44	192	4	6
Grand mean ±SE	62±3	262±42	724±94	166±26	99±19

* Average value±SE of samples taken during the final hour of study except for single values, which were taken at the end or at the beginning of the study.

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ium hydroxide, and the proteins were precipitated by subsequent addition of 0.5 ml of 0.3 N zinc sulfate. After centrifugation, the supernatant was used for determination of isotopic enrichment in plasma glucose and glycerol as described below. In each of the glycerol studies, a single additional 200-μl blood sample was obtained at the end of the study and prepared for plasma FFA analysis by extraction of the plasma into Dole's mixture as described earlier.

Analytical methods. Plasma glucose (16), glycerol (17), and β-hydroxybutyrate (18) were measured microfluometrically by specific enzymatic analyses. Plasma palmitate content and [1-^{13}C]palmitate enrichment were measured by selected ion monitoring and isotope dilution GCMS as recently described (19). Briefly, FFA isolated from the Dole's mixture extract (20) were converted to methyl ester derivatives by treatment with 1% H_2SO_4 in methanol. The resultant individual fatty acid methyl esters were resolved by gas chromatography (GC) as described (15, 19). Computer-controlled, electron impact, selected ion monitoring mass spectrometry (MS) (19) of the palmitic acid molecular ion cluster (m/z 270, 271, and 274) representing unlabeled, tracer [1-^{13}C], and internal standard [5,5,6,6-^{2}H$_4$]palmitic acid, respectively, allowed subsequent calculation of plasma palmitate content and [1-^{13}C]palmitate tracer enrichment (19). Plasma total FFA content was calculated from the summed individual fatty acid GC peak areas referenced to the palmitate content (15). Total plasma FFA calculated in this fashion is somewhat less than the value obtained by titration because the latter method also measures fatty acids of shorter chain length than those determined by GC analysis.

The $Ba(OH)_2$-$ZnSO_4$ plasma supernatant was subjected to sequential anion and cation exchange chromatography (21) and 100 μl of the neutral eluate fraction was used to measure plasma [6,6-^{2}H$_2$]glucose enrichment by selected ion monitoring GCMS as described (21). Plasma [^{13}C]glucose (formed from [2-^{13}C]glycerol) was measured in the same assay by measuring the M + 1 ion as well. The latter was corrected for the natural isotopic contributions of the other glucose molecular species present in the ion cluster by standard mass spectrometric isotopic dilution equations equivalent in form to those previously presented for palmitate (19). The remainder of the ion exchange eluate was dried under N_2 and the glycerol -1,2,3-triacetate ester prepared by addition of 20 μl of acetic anhydride and 10 μl of pyridine. The reaction was carried out at room temperature for 1 h. Gas chromatographic resolution of glycerol triacetate was accomplished on a 2 m $\times$ 2 mm 3% OV17 column maintained at 115°C, and [2-^{13}C]glycerol isotopic enrichment measured by electron impact, selected ion monitoring MS of the [M-CH$_2$OCOCH$_3$]$^+$ ion (m/z 145) reflecting unlabeled glycerol, and its corresponding M + 1 ion (m/z 146) representing carbon-13-labeled glycerol (after correction for natural ^{13}C background). In representative samples, methane chemical ionization and selected ion monitoring GCMS of the MH$^+$ ion cluster (m/z 219 and 220) produced results identical to those obtained by electron impact analysis.

Calculations. Plasma palmitate inflow transport (or rate of appearance, R_a), its rate of outflow (or disappearance, R_d), and its clearance rate (R_c) were calculated from samples taken between 60 and 120 min of study as described (15). For this purpose, the volume of distribution of palmitate was equated with the plasma volume estimated from the data of Cassidy (22). The fractional removal rate of palmitate was calculated by dividing the outflow rate by the palmitate pool size (taken as plasma concentration $\times$ plasma volume). In three of the group A newborns, plasma palmitate content and isotopic enrichment remained reasonably constant (SD/

mean < 0.10) during the final 60 min of study and thus could be considered at or near steady state where $R_a = R_d =$ flux, a specialized case for the equations noted (15). Total FFA inflow and outflow transport rates were calculated from palmitate's fraction of total circulating FFA as described (15) because palmitate is a representative tracer for circulating fatty acids as a group (23).

In group B, each subject achieved approximately constant (SD/mean < 0.11) values for plasma glucose and glycerol content, [6,6-^{2}H$_2$]glucose enrichment, [1-^{13}C]glucose enrichment, and [2-^{13}C]glycerol enrichment during the latter 30–45 min of study. Thus, glucose and glycerol flux ($R_a = R_d$) were calculated by conventional steady-state tracer dilution equations (1). Using these values, the appearance rate of [^{13}C]glucose and the rate of glycerol conversion to glucose were estimated by standard tracer precursor-product approaches (24).

RESULTS

Substrate levels. Plasma substrate levels in the studied infants are shown in Table II. None of the infants was hypoglycemic. Plasma palmitate averaged 262±42 μM. Because palmitate represented 36.2±2.1% of the circulating long chain FFA, a newborn value similar to that reported by others (25), total circulating FFA averaged 724±94 μM. This level is in the range previously reported for newborns on the first day of life (5–7, 26). The intersubject variability of plasma FFA was presumably due to the wide postprandial range of sampling in our infants whose plasma FFA levels were correlated directly with the time since their last feeding ($r = 0.57$, $P = 0.02$). 8 of 11 subjects in group A also showed broad intrasubject variability in plasma palmitate and FFA levels. In only four of these infants, however, were the variations systematic. In patient 3, plasma palmitate declined from 388 to 134 μM during the last hour of study. In patients 7, 8, and 9, plasma palmitate rose from 84 to 154 μM, from 89 to 209 μM, and from 384 to 515 μM, respectively, during the last 60 min of study. These changes reflected the unequal plasma inflow and outflow rates (Table III). Likewise, there was wide intersubject variability in plasma glycerol and β-hydroxybutyrate levels, but these values were in the range reported for infants (5, 26), and both correlated significantly ($P < 0.001$) with the corresponding FFA concentrations (Fig. 1). The ratio of circulating glycerol to FFA averaged 0.25±0.04, higher than the average value of $\sim$0.1 found by Novak et al. (5), but within the range reported by Heim (27), Persson and Gentz (26), and Melichar and Wolf (28) for infants during the first days of life.

Palmitate and FFA transport. Table III presents the results of the [1-^{13}C]palmitate tracer infusion study in group A. During the study, infused [1-^{13}C]tracer reached an average isotope enrichment of 1.38 atom % excess. Palmitate (and consequently FFA) inflow

TABLE III
FFA Kinetics in the Newborns Studied

Subject	Plasma [1-^{13}C] palmitate	Palmitate R_a	Palmitate R_d	FFA R_a	FFA R_d
	atom % excess	$\mu mol\ kg^{-1}\ min^{-1}$		$\mu mol\ kg^{-1}\ min^{-1}$	
1	0.79±0.09	5.3±0.2	5.2±0.2	12.7	12.5
2	1.73±0.16	5.4±0.3	5.4±0.3	13.4	13.4
3	0.86±0.09	7.7±0.8	7.9±0.7	25.5	26.2
4	2.16±0.10	2.6±0.1	2.6±0.1	7.4	7.4
5	0.81±0.03	8.7±0.3	8.7±0.4	20.9	20.9
6	1.61±0.07	3.9±0.06	3.9±0.06	13.4	13.4
7	1.91±0.03	2.8±0.03	2.7±0.03	7.6	7.3
8	1.37±0.21	3.2±0.5	3.1±0.4	10.3	9.9
9	0.78±0.12	10.1±0.4	9.7±0.01	18.2	17.5
10	1.83±0.08	3.6±0.1	3.5±0.1	12.1	11.9
11	1.33±0.09	5.0±0.2	4.9±0.2	11.1	11.0
Mean	1.38	5.30	5.25	13.9	13.8
SE	0.15	0.75	0.75	1.7	1.7

and outflow transport rates were identical or nearly so in almost all subjects. Note, however, that palmitate inflow–outflow discrepancy of only 0.2–0.3 μmol $kg^{-1}min^{-1}$ can lead to a substantial plasma palmitate change if maintained over a 60-min period as noted above, particularly for subject 3. As in the adult (15, 29–36), there was a close relationship ($r = 0.84$, $P < 0.001$) between plasma palmitate level and palmitate inflow rate (Fig. 2). Similarly, palmitate inflow
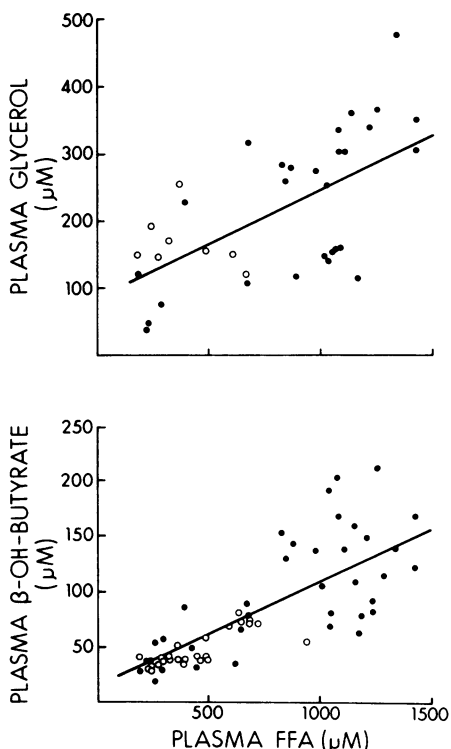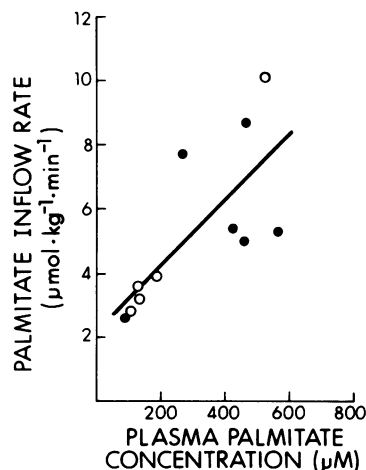

FIGURE 1 Relationships of plasma glycerol (upper panel) and β-hydroxybutyrate (lower panel) to plasma FFA. The closed circles are individual values from term infants and the open circles are from preterm infants. The regression line in the upper panel is defined by the equation Y = 0.16X+84.7 ($r = 0.62$, $P < 0.001$) and the line in the lower panel by the function Y = 0.096X+13.67 ($r = 0.78$, $P < 0.001$).

FIGURE 2 Relationship between average plasma palmitate level and palmitate inflow transport during the last 60 min of [1-^{13}C]palmitate tracer infusion in group A subjects. The closed circles are values from term and the open circles values from preterm infants. The regressive equation is Y = 0.010X+2.4 ($r = 0.73$, $P < 0.01$).

correlated directly ($r = 0.85$, $P < 0.001$) with plasma glycerol level as expected from the consequences of lipolysis of stored or dietary triglycerides.

The average FFA inflow rate was 13.9 ± 1.7 μmol kg^{-1}min^{-1}. For the purposes of comparison, the infants were divided arbitrarily into two groups on the basis of feeding history. In six subjects (Nos. 4 and 6–10) FFA transport was calculated during a period <4–4.5 h after the last feeding, a common intermeal interval for the human newborn. FFA inflow in these subjects averaged 11.5 ± 1.7 μmol kg^{-1}min^{-1}, a value comparable to that found in postabsorptive adults 8–12 h after their last meal (15, 33, 37–39). In the five remaining infants who were not fed for >5 h before FFA transport was calculated, FFA inflow averaged 16.7 ± 2.8 μmol kg^{-1}min^{-1}, a value not reached in adults until they are fasted for 3 d or more (38, 39).

Plasma palmitate clearance ranged from 9 to 33 ml/ min (average 19.6 ± 2.4 ml/min) and was negatively correlated ($P < 0.002$) with plasma palmitate concentration in the range of 80 to 560 μM. Likewise (because clearance and fractional removal are related through a constant) fractional removal was also highly inversely correlated with plasma palmitate concentration (Fig. 3) as in the adult (37). The fractional rate constant declined from 0.6 to 0.2/min over a plasma palmitate range of 80 to 560 μM (equivalent to an FFA range of ~250 to 1,500 μM), values remarkably similar to adult palmitate and FFA fractional removal at comparable FFA levels (30, 37, 40, 41). Hagenfeldt (30) was concerned that this inverse correlation might actually reflect an effect of the interindividual relationship between FFA and body weight. However, in subject 3, who had the widest range of plasma palmitate during the course of the study, fractional removal was

highly inversely correlated with palmitate level ($r = 0.99$).

Glycerol metabolism. Glycerol flux in the group B infants averaged 4.4 ± 0.5 μmol kg^{-1}min^{-1}, a value three- to fourfold that of postabsorptive adults (32, 38, 42) despite the fact that the infants were fasted only 4–6 h. Of the circulating glycerol, 3.3 ± 0.6 μmol kg^{-1}min^{-1}, or ~75% of the transported substrate, was converted to glucose. Because simultaneous glucose flux averaged 32.2 ± 3.4 μmol kg^{-1}min^{-1} (5.8 ± 0.6 mg kg^{-1}min^{-1}), a normal newborn value (1), glycerol contributed $5.0\pm0.6\%$ of hepatic glucose production under the conditions of this study. If all the circulating glycerol had been converted to glucose it would have been responsible for ~7% of glucose output. Despite the small number of subjects and the relatively narrow range of glycerol and glucose turnover rates, there was a significant ($r = 0.85$, $P = 0.03$) direct relationship between glycerol transport and the fraction of glucose production coming from glycerol (Fig. 4). Bortz et al. (42) described a sigmoidal relationship between these two variables in the adult. Their curve, however, was "drawn by eye" and a linear fit appears equally plausible for their displayed data.

From the group B infants' FFA values (Table II) and the regression equation previously defined for the infants of group A (Fig. 2), the estimated FFA inflow for the group B infants was 12.8 ± 1.9 μmol kg^{-1}min^{-1}, in good agreement with the value of 11.5 ± 1.7 μmol kg^{-1}min^{-1} found in group A infants fasted for a similar period of time. Furthermore, the ratio of FFA transport to glycerol transport averaged 2.88 ± 0.17, close to the theoretical value of 3.0 expected if none of the hydrolyzed triglyceride fatty acid was reesterified in adipose tissue without release into the circulation.

DISCUSSION

Despite the obvious importance of lipid fuel for maintenance of energy balance in the newborn, we know of no direct measurements of FFA or glycerol transport in this age group. Our study demonstrates that FFA mobilization from triglyceride stores is a very active process during the first day of life even for moderately preterm infants. The observed neonatal FFA inflow transport rates of 7.4–13.4 μmol kg^{-1}min^{-1} in 8 of the 11 infants agree remarkably well with the FFA turnover of 4.9–13.4 μmol kg^{-1}min^{-1} found in four control children and four children recovered from Kwashiorkor, aged 8 mo–3 yr 8 mo, studied with [1-^{14}C]palmitic acid (43), the only other pediatric FFA flux data of which we are aware. Three of our infants exceeded these values. Direct comparison with the adult is difficult because data in the latter group are generally obtained in the postabsorptive state after an
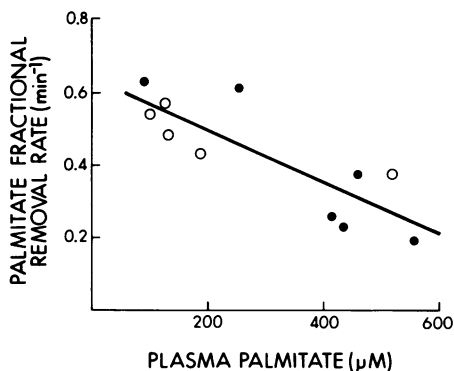

FIGURE 3 Relationship between average plasma palmitate content and fractional removal rate during the last 60 min of [1-^{13}C]palmitate tracer infusion. The closed circles are term and the open circles preterm infants. The regression is defined by the equation $Y = -7.13 \times 10^{-4}X + 0.64$ ($r = -0.83$, $P < 0.001$).
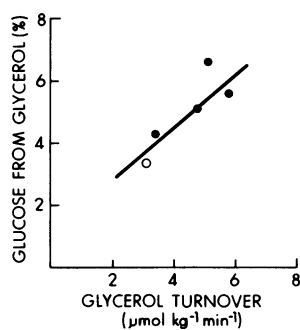
FIGURE 4 Relationship between glycerol transport and the fraction of glucose production coming from glycerol. Closed circles are term infants and the open circle is a preterm infant. The equation for the line is $Y = 0.93X+0.86$ ($r = 0.85$, $P = 0.03$).

8–12-h fast. However, our neonatal FFA turnover rates were also greater than those found in the adult by Nestel et al. (41) 6–8 h after discontinuation of sustained glucose consumption. Furthermore, it is of interest that newborns fasted for only 4 h or so have FFA flux rates comparable to those found in postabsorptive adults, while infants fasted for 5–12 h have FFA inflow transport rates achieved by the adult only after several days of fasting.

Because the newborn is generally fed on an approximately-every-4-h basis, one might estimate the total daily fatty acid transport from the group A infants who were studied within 5 h of their last feeding. From the observed average FFA inflow transport rate of 11.5 ± 1.7 μmol kg^{-1}min^{-1}, total daily FFA transport is on the order of 4–5 g kg^{-1}d^{-1}. In the adult, $\sim$30% of FFA turnover is oxidized (36, 39). If one assumes the same fractional oxidation rate for the newborn (because such data are not yet available) the neonate would oxidize 1.2–1.5 g of fat per kilogram body weight daily, equivalent to 11–13.5 kcal kg^{-1}d^{-1}. The latter values represent one-fourth to one-third of the minimal energy requirements of the newborn on the first days of life (2). This range agrees well with respiratory quotient estimates of lipid contribution to daily energy expenditures in newborns receiving oral or intravenous nutrients but reflects only one-half to one-third of the lipid's contribution to total energy metabolism in unfed neonates (27, 44–47).

As in the adult (29–37), fatty acid levels in our newborns were directly correlated with inflow transport (Fig. 2) and inversely correlated with fractional removal (Fig. 4). Furthermore, there was general similarity between neonatal and adult fractional removal rates at different FFA levels, as well as close agreement between the regression equation for FFA content and inflow transport in our patients and the average regression calculated from data presented in eight adult studies (15, 29–35) (Fig. 5). Although it was not possible

to accurately calculate the variance about the latter adult regression line because all of the individual values are not known, it seems clear from the data points for our newborn regression equation (Fig. 2) that the two lines in Fig. 5 are unlikely to be statistically different. Taken together, the similar adult and newborn relationships between FFA concentrations and kinetic parameters suggest that the regulatory systems controlling these relationships are already fully established in man on the first day of life.

As expected from the above FFA data and from the calculated FFA/glycerol ratio near 3.0, neonatal glycerol transport related to available adult glycerol turnover information in much the same fashion as did the FFA data. Thus, despite a relatively short fast, neonatal glycerol turnover was severalfold that of the postabsorptive adult (32, 38, 42). Nevertheless, because the average neonatal glycerol flux of 4.4 μmol kg^{-1}min^{-1} represents transport of only $\sim$0.6 g kg^{-1}d^{-1}, glycerol is unlikely to be a major fuel source under usual circumstances.

Glycerol's other role of gluconeogenic precursor, however, requires discussion. In this study, 75% of the transported glycerol was converted to glucose. That the bulk of glycerol turnover becomes new glucose is consistent with the value of 40–80% glycerol carbon converted to glucose carbon in the lean adult (42), 50% in newborn pups (48), and $\sim$70% in the rat (49). In our infants, the 3.3 ± 0.6 μmol kg^{-1}min^{-1} of glycerol that became glucose represented 5% of glucose output, a contribution similar to the range of 1.9–5.2% found in postabsorptive adults (42). Thus, glycerol's gluconeogenic contribution is about half that of alanine's in infants <8 h of age (50). Hall et al. (51) also found
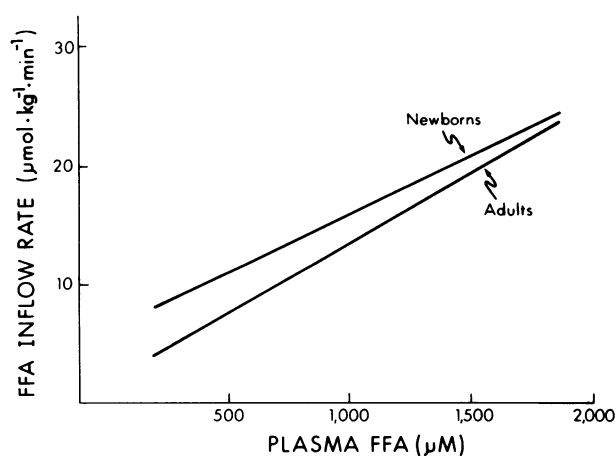

FIGURE 5 Relationships between plasma FFA content and FFA inflow transport in the newborn (this study) and in the adult (mean of values calculated from individual regression equations given in references 15, 29–35). The newborn line is defined by the equation $Y = 0.010X+6.0$ and the adult average function is $Y = 0.012X+1.0$.

TABLE IV

Glucose and Glycerol Kinetics and the Conversion of Glycerol to Glucose in the Newborns Studied

Subject	[1-^{13}C] Glycerol	[6,6-^{2}H$_2$] Glucose	[^{13}C] Glucose	Glycerol flux	Glucose flux	Glycerol to glucose conversion	Fraction of glycerol converted to glucose	Fraction of glucose coming from glycerol	Maximal contribution of glycerol to glucose‡
		atom % excess			μmol kg^{-1} min^{-1}		%	%	%
12	11*±0.4	0.69±0.02	0.90±0.01	3.4	35.6	3.1	91	4.3	4.8
13	7.7±0.1	0.92±0.06	0.52±0.02	3.1	22.8	1.5	48	3.3	6.8
14	5.8±0.1	0.73±0.02	0.77±0.01	5.1	32.4	4.3	84	6.6	7.9
15	5.0±0.1	0.5±0.01	0.64±0.02	5.8	42.0	4.7	81	5.6	6.9
16	5.9±0.1	0.95±0.03	0.60±0.01	4.8	28.4	2.9	60	5.1	8.4
Mean	7.1	0.76	0.69	4.4	32.2	3.3	72.8	5.0	7.0
SE	1.1	0.08	0.07	0.5	3.2	0.6	8.1	0.6	0.6

* Infant 12 was infused with a significantly higher dose of [2-^{13}C]glycerol (see text).
‡ If all glycerol turnover were converted to glucose.

that glycerol's contribution to glucose production was about half that of alanine in newborn dogs. But, in their study, both glycerol and alanine each supplied approximately twice the fraction of glucose carbon than the respective values found in the human infant.

In the human, FFA values rarely exceed 1.5–2.0 mM in the neonatal period (5–7, 26–28). From Fig. 5 and from an FFA/glycerol transport ratio of nearly 3, one can estimate that neonatal glycerol flux is unlikely to surpass 7–8 μmol kg^{-1}min^{-1}. Consequently, we should not expect glycerol to supply >~8% of neonatal hepatic glucose output (Fig. 4). Similarly, while tracer techniques underestimate actual gluconeogenic rates (52), the maximum contribution of glycerol to glucose production in our infants would have been 7% if all transported glycerol were converted to glucose (Table IV).

Nevertheless, a 5–8% contribution to glucose production can be critically important to the newborn. A 3.5-kg term newborn produces ~20 mg of glucose/ min (1), just about the glucose requirement of the 400-g newborn brain (1). This precarious fuel balance could be disturbed easily by a reduction in glycerol's contribution to glucose production if not matched by a simultaneous increase in delivery of other gluconeogenic substrate, by an exogenous source of glucose, or by the glucose-sparing effect of increased utilization of alternative fuels such as ketone bodies. Thus, as expected, accelerated lipid mobilization in the newborn serves a dual fuel balance role: delivery of substrate both for energy and for gluconeogenesis.

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