Determination of ketone body kinetics using a D-(−)-3-hydroxy[4,4,4-$^2H_3$]butyrate tracer

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Abstract In studies where D-(−)-3-hydroxy[4,4,4-$^2H_3$]butyrate is employed as isotopic tracer in vivo, we have described a selected ion monitoring, gas-liquid chromatography-mass spectrometry micromethod which measures [$^2H_3$] tracer enrichment in 3-hydroxybutyrate and acetoacetate from 300-μl blood samples. For plasma samples in the physiologic range, intra- and interassay precisions for each ketone averaged better than ± 1% and ± 2%, respectively. The use of the method was validated by comparing kinetic data obtained with the above tracer with simultaneous flux data obtained with conventional D-(−)-3-hydroxy[3-^{14}C]butyrate tracer in five fasted rats. —**Bougneres, P. F., E. O. Balasse, P. Ferré, and D. M. Bier.** Determination of ketone body kinetics using a D-(−)-3-hydroxy[4,4,4-$^2H_3$]butyrate tracer. *J. Lipid Res.* 1986. **27:** 215–220.

Supplementary key words deuterium • ketones • acetoacetate • 3-hydroxybutyrate • ketone body turnover • stable isotopes

The ketone bodies, acetoacetate and 3-hydroxybutyrate, are important metabolic fuels in infancy (1–3), and can replace glucose as an energy source for the central nervous system (3–8). However, despite the fact that hyperketomenia develops early in neonatal life (9–12), ketone body transport has not yet been quantified in the human newborn. In large part, this deficiency has been due to the lack of a suitable approach to this measurement, since at least two requirements have to be satisfied. *1*) The isotope dilution method must be non-invasive, adaptable to very small blood samples, and ethically acceptable for a neonatal study; and *2*) it must allow assessment of tracer enrichment both in 3-hydroxybutyrate (3-OHB) and in acetoacetate (AcAc), since both values are necessary for subsequent calculation of total ketone body flux.

In this report, we present a new method which satisfies the above requirements. Non-radioactive D-(−)-3-hydroxy[4,4,4-$^2H_3$]butyrate tracer ($^2H_3$-OHB) is used and $^2H_3$ isotopic enrichment is determined simultaneously both in blood 3-OHB and in AcAc using selected ion monitoring gas-liquid chromatography-mass spectrometry (GLC-MS). The method is simple, reliable, and requires only 200–300 microliters of blood which can be obtained by "heel stick" sampling.

MATERIALS AND METHODS

Materials

Sodium D,L-3-hydroxybutyrate (analytical grade) and lithium acetoacetate (90–95% purity), as well as the other reagents required for spectrophotometric enzymatic assays of 3-OHB and AcAc concentration (13) were obtained from the Sigma Chemical Company (St. Louis, MO). D-(−)-3-hydroxy[3-^{14}C]butyrate, specific activity 50 mCi/mmol (^{14}C-OHB), was purchased from Amersham Searle (Arlington Heights, IL). Sodium D-(−)-3-hydroxy[4,4,4-$^2H_3$]butyrate (91 atom % $^2H_3$) was purchased from Merck Isotopes (Dorval, Quebec). Its chemical purity was confirmed both by spectrophotometric enzymatic analysis (13) and by inverse isotope dilution using selected ion monitoring GLC-MS (see below). Agreement between chemical purity measured using the former assay, which is specific for D-(−)-3-hydroxybutyrate, and the latter dilution assay, which does not discriminate between the D and L stereoisomers, indicated that the tracer was not contaminated with L-3-hydroxybutyrate.

Stock solutions of unlabeled AcAc, unlabeled 3-OHB, and [$^2H_3$]OHB were prepared by dissolving 5–10 mg of accurately weighed ketone in 10 ml of bi-distilled water. Working calibration standard solutions were prepared by serially diluting various combinations of labeled and

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Abbreviations: 3-OHB, 3-hydroxybutyrate; AcAc, acetoacetate; GLC-MS, gas-liquid chromatography-mass spectrometry; TMS, trimethylsilyl.

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unlabeled 3-OHB stock solutions (see results) which were kept stored briefly at −20°C.

Since a [4,4,4-$^2H_3$]acetoacetate ($^2H_3$-AcAc) standard was not commercially available, it was prepared by enzymatic oxidation of D-(−)-3-hydroxy[4,4,4-$^2H_3$]butyrate (13). The precursor [$^2H_3$]OHB and the product [$^2H_3$]AcAc were analyzed as their trimethylsilyl derivatives (see below). Full sweep mass spectra were identical to published reference spectra (14, 15) except for the appropriate mass shifts due to the presence of deuterium. The isotopic enrichment (91.14 atom % $^2H_3$) of the [$^2H_3$]AcAc generated enzymatically was identical to that of the precursor [$^2H_3$]OHB (91.19 atom % $^2H_3$).

The [$^2H_3$]AcAc was stored frozen for 3 days. When reanalyzed by selected ion monitoring GLC-MS, the relative ion current values (normalized to the molecular ions (M+) m/z 234 for TMS-[$^2H_3$]AcAc and m/z 236 for TMS-[$^2H_3$]OHB) were as follows.

	M−3	M−2	M−1	M+	M+1	M+2	M+3
[$^2H_3$]OHB	6.34	3.19	2.29	100	20.42	9.26	1.27
[$^2H_3$]AcAc	6.04	3.20	2.23	100	20.45	10.06	1.97

These data indicated that there was no loss of deuterium from [$^2H_3$]AcAc under the storage conditions reported.

Furthermore, we monitored the deuterons at position 4 of [$^2H_3$]AcAc in water for a period of 4 hr by magnetic resonance spectroscopy and were unable to detect exchange of these deuterons with aqueous protons. Likewise, in a complementary experiment, there was no detectable exchange of deuterons for protons in natural acetoacetate dissolved in D_2O when monitored by NMR for 4 hr.

To assess potential for in vivo exchange prior to the validation experiments with [^{14}C]ketone tracer reported below, a rat was infused with [$^2H_3$]OHB tracer for 90 min. Prior to this infusion, the m/z 236/233 ion current ratio in plasma 3-OHB analyzed as the TMS derivative was 1.06%. At 60, 75, and 90 min the ratio was 2.53, 2.33, and 2.56%, respectively. The corresponding ion current ratios in circulating acetoacetate measured as the TMS derivative were as follows.

Ion ratio	0 min	60 min	75 min	90 min
232/231	20.25	20.40	20.40	20.31
233/231	7.04	7.07	7.10	7.03
234/231	0.81	1.75	1.67	1.75
235/231	0.14	0.14	0.16	0.15

The constant 234/231, 233/231, and 232/231 ion current ratios for 90 min in vivo support the conclusions of the in vitro studies above that the loss of one or two deuterons from [$^2H_3$]AcAc was insignificant during the time-frame of the experiments conducted later (vide infra).

For in vivo studies, the desired amount of sodium [$^2H_3$]OHB was diluted in normal saline and passed through a 0.22 μm Millipore® filter into sterile vials for infusion. An aliquot was determined to be pyrogen-free by conventional rabbit body temperature assay in a licensed commercial laboratory. The actual [4,4,4-$^2H_3$]AcAc concentration in stock solutions was verified by spectrophotometric assay on the day these solutions were used to establish calibration curves.

Procedural methods

The ability of the method to accurately estimate ketone body flux in vivo was evaluated by studies in five anesthetized, overnight-fasted adult Wistar rats (200–250 g). A 25-gauge catheter was placed in the carotid artery for blood sampling. Two tracers were infused simultaneously via a 25-gauge catheter which had been inserted into a forelimb vein: [^{14}C]OHB was administered at the nominal rate of 300,000 dpm/min. [$^2H_3$]OHB was infused simultaneously at the nominal rate of 0.5 μmol/min. Sixty minutes were allowed to achieve isotopic steady state. The animals were then bled through the arterial catheter, and the blood was divided into three aliquots for determination of *a*) AcAc and 3-OHB concentrations (0.1 ml) (13); *b*) $^2H_3$ enrichment (0.3 ml), see below; and *c*) ^{14}C specific activities (2 ml) (16). The administration rate of the nonradioactive [$^2H_3$]OHB tracer was confirmed by direct spectrophotometric enzymatic analysis of the infusate. The infusate rate of [^{14}C]OHB was determined by multiplying the calibrated rate of infusion (ml/min) by the ^{14}C radioactivity per milliliter of the infusate. Total ketone body appearance rates were calculated utilizing radiotracer steady-state dilution equations (17) or their equivalent for stable isotopically labeled tracers (18).

Analytical methods

In order to extract ketone bodies for GLC-MS analysis, 0.3 ml of blood was added immediately after collection to 0.5 ml of 1 M perchloric acid in iced tubes and mixed thoroughly. The mixture was then centrifuged at 4°C for 5 min. The supernatant was transferred to another iced tube and the pH was immediately adjusted to 6.5–7.5 with 10% KOH. The potassium perchlorate salts were sedimented by another 5-min centrifugation at 4°C. The supernatant was transferred to another cold tube and reacidified on ice with two drops of 1 N HC1. The regenerated ketoacids were extracted twice into 1 ml of ethyl acetate, the combined extracts were pooled in a 2.5-ml conical microreaction vial, and the solvent was evaporated to dryness under dry N_2 at room temperature. The residues were resuspended in 30 μl of 10% pyridine in BSTFA (Pierce Chemical, Rockford, IL), and heated at 60°C for 30 min to prepare the trimethylsilyl esters. In this form, samples are stable and isotopic enrichment

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values are constant for periods of at least 5 days when stored at −80°C.

3-OHB and AcAc trimethylsilyl ester separation from other plasma trimethylsilyl esters was accomplished by 120°C isothermal gas-liquid chromatography on a 2 m × 2 mm glass column packed with 3% OV1 on Supelcoport 100/200 mesh using helium carrier gas. Under the GLC conditions used, D and L enantiomers of 3-OHB cochromatograph and the L-3-OHB (from the racemic mixture used to prepare the standards) has the same mass spectrum as its D counterpart. The ratios of labeled to unlabeled 3-OHB and AcAc were then quantified by electron-impact, selected ion monitoring GLC-MS using an R10-10 Nermag Mass Spectrometer (Rueil Malmaison, France) equipped with an Incos 200 Data System, Finnigan Mat (Sunnyvale, CA). For measurement of ion current ratios in the range reported here, this instrument system has a relative precision of ± 0.5% of the measured ratio.

Mass spectrometer ion source temperature was maintained at 200°C and ionization voltage at 70 eV. Ions at m/z 233 and 236, representing the $(M-15)^+$ ions of the unlabeled and the $[^2H_3]$OHB trimethylsilyl esters, and ions at m/z 231 and 234, representing the unlabeled and the $[^2H_3)$AcAc trimethylsilyl esters, respectively, were selectively monitored using a mass-calibrated, multiple ion monitoring program. The corresponding peak areas were integrated by the data system and $^2H_3$ enrichments of 3-OHB and AcAc were calculated from these data, corrected for the slope of a standard curve of known $[^2H_3]$AcAc and $[^2H_3]$OHB mixtures measured in the same analytical run (see below). In any case, with a properly tuned mass spectrometer, the slope of this standard curve was not statistically different from 1.0. Since each analytical run requires injection of only approximately 2–4 µl of the final pyridine-BSTFA solution, several replicate measurements can be made on each 0.3-ml blood sample. Blood AcAc and 3-OHB concentrations (13) and specific activities (16) were determined as described above.

Calculations

Prior to blood analysis, calibration standards containing the same amount of sodium D,L-3-OHB but different amounts of sodium $[^2H_3]$OHB were analyzed by selected ion monitoring GLC-MS to establish a standard curve defined by the equation

$$Rc = Ro + k\,(n3/n0) \qquad \textit{Eq. 1)}$$

where Rc is the observed 236/233 ion current ratio of the calibration standard; Ro is the natural 236/233 ion current ratio; n0 and n3 are the absolute amounts (in moles) of natural 3-OHB and D-(−)-3-hydroxy[4,4,4-$^2H_3$]butyrate, respectively, in the calibration standard; and k is the slope of 236/233 ion current ratio as a function of n3/n0. From the above determined slope (k), the atom % $^2H_3$ enrichment of a sample (Es) can be calculated from the experimentally observed 236/233 ion current ratio in the tracer-enriched sample (Rs) and in the pre-infusion sample (Ro) as follows:

$$Rs = 1/k\,(Rs - Ro) \qquad \textit{Eq. 2)}$$

$$Es = Rs/1 + Rs \times 100 \qquad \textit{Eq. 3)}$$

The same isotope dilution technique was used to define a calibration curve equivalent to equation 1 by GLC-MS analysis of calibration standards containing the same amount of natural lithium acetoacetate and different amounts of [4,4,4-$^2H_3$]acetoacetate prepared as described previously.

$$R'c - R'o + k'(n'3/n'O) \qquad \textit{Eq. 4)}$$

Where R'c is the observed 234/231 ion current ratio of the calibration standard; R'o is the natural 234/231 ion current ratio; n'o is the absolute amount (in moles) of natural AcAc, n'3 is the measured amount of $[^2H_3]$AcAc added in the calibration standard; and k' is the slope of 234/231 ion current ratio as a function of n'3/n'O. Then the atom % $[^2H_3]$AcAc enrichment, Es, can be calculated using equations 2 and 3.

RESULTS

To test the linearity of the assay (**Fig. 1**), a series of standard solutions containing 30–600 nmol of 3-hydroxybutyrate (equivalent to the content of 0.3 ml of blood containing 3-OHB in the physiological range of 0.1–2.0 mM) and 3 nmol of D-(−)-3-hydroxy[4,4,4-$^2H_3$]butyrate

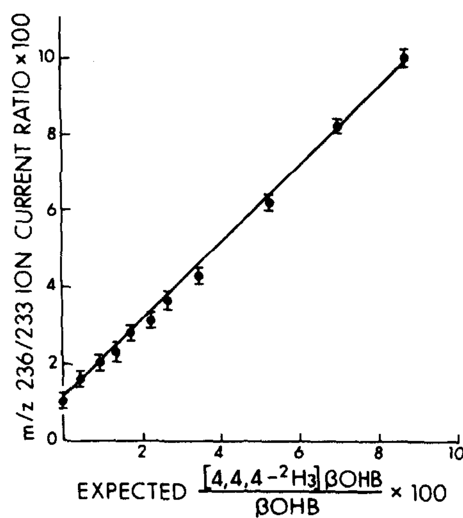

Fig. 1. Calibration curve for mixtures of natural and D-(−)-3-hydroxy[4,4,4-$^2H_3$]butyrate produced by addition of known amounts of natural 3-OHB to a $[^2H_3]$OHB standard solution. The equation for the line is Y = 1.03 x + 1.00; r = 0.998.

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were prepared. Quadruplicate stock solutions were prepared by separate, individual weighings in 10 ml of water as noted above. Quadruplicate sets of working standard were prepared therefrom by serial dilution on 0.3 ml of bidistilled water. The samples were analyzed by GLC-MS as described. Fig. 1 shows that the assay was linear throughout the measured range.

The average precision (SD/mean × 100) of ion current ratio measurement was ± 4%. Likewise, assay of a parallel series of standards containing 30-600 nmol of AcAc and 3 nmol of [4,4,4-$^2H_3$]AcAc analyzed in a similar fashion (**Fig. 2**) was also linear with an average relative precision of ± 5%. This coefficient of variation, therefore, represents that of the total propagated errors: weighing, pipetting, dilution, and instrument noise. For repeated measurements of a given standard solution, the relative precision was 0.5%, equivalent to the instrument precision for measuring ion current ratios in general as noted above in Analytical Methods.

Intra-assay precision (SD/x × 100), assessed by quadruplicate measurement of each of four plasma samples containing [$^2H_3$]OHB and [$^2H_3$]AcAc in the 0.6-1.8 atom % excess range, was ± 0.7% for [$^2H_3$]OHB and ± 0.9% for [$^2H_3$]AcAc, respectively. Inter-assay precision, quantified in an identical fashion for similar plasma samples analyzed on three separate occasions, averaged ± 1.8% for each labeled ketone body.

Table 1 shows the results of the in vivo validation study carried out in rats as described above. The circulating total ketone body concentration averaged 2.83 mM of which two-thirds was 3-hydroxybutyrate. The specific activity of [3-^{14}C]AcAc averaged 79.3 ± 4.5% of the [3-^{14}C]OHB specific activity, a value not different from the 76.1 ± 10.3% relative isotopic enrichment of [$^2H_3$]AcAc compared with [$^2H_3$]OHB. Similar relative values have been reported in rats by others (19, 20). The approximate equivalency in isotopic distribution between AcAc and 3-OHB in rats provides the rationale for the use of total ketone body isotopic enrichment (E_{kb}) or specific activity (SA_{kb}) for calculation of total ketone turnover by the "single pool" approach (20, 21). Thus, when calculated from the combined [3-^{14}C]ketone specific activity (SA_{kb}), total ketone turnover averaged 17.3 ± 6.2 μmol/min, a value virtually identical to the rate of 17.9 ± 4.4 μmol/min calculated from the combined [$^2H_3$]ketone enrichment (E_{kb}).

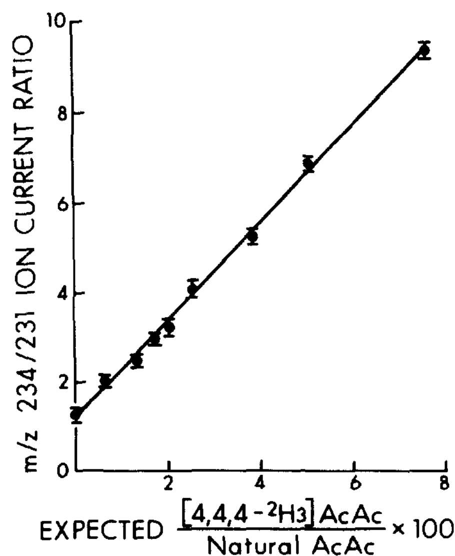

Fig. 2. Calibration curve for mixtures of natural and [4,4,4-$^2H_3$]acetoacetate produced by addition of known amounts of natural AcAc to a [$^2H_3$]AcAc standard solution. The equation for the line is Y = 1.08 × + 1.24; r = 0.999.

DISCUSSION

Currently available methods for the determination of plasma ketone body inflow kinetics in vivo include either invasive measurement of arteriovenous stoichiometry across the splanchnic bed or quantitation of ^{14}C-labeled ketone body tracer dilution. Neither of these approaches satisfies the ethical constraints necessary for investigation of ketone body metabolism in certain human groups, notably infants and children. Furthermore, the measurement of ^{14}C specific activity in circulating ketone bodies is not only modestly difficult, but also requires separate measurements of ketone content in addition to the determination of radioactivity in the ketones. Thus, because of greater potential for propagated errors, variance in calculated specific activity is likely to be more than that for stable isotope enrichment measurements where the labeled and unlabeled substrates are determined simultaneously by the same method.

Conversely, the use of the tracer [$^2H_3$]OHB and the GLC-MS determination of $^2H_3$ enrichment both in 3-OHB and in AcAc from microliter plasma samples provides a useful approach not only for measurement of ketone body flux in newborn infants, children, and pregnant women, but also for determination of ketone turnover in small animals via sequential sampling. The excellent agreement between the [$^2H_3$]OHB and [^{14}C]OHB isotopically determined rates of ketone body appearance indicate that both tracers quantify the same in vivo kinetic phenomena, and that the deuterons substituted on carbon 4 are conserved in the reversible interconversion between 3-OHB and AcAc in vivo. The latter conclusion is supported by our direct magnetic resonance observations of the course of the 3-hydroxybutyrate dehydrogenase reaction in vitro, and by the in vivo studies of Reed et al. (19), who showed isotopic equivalence for [3-^{14}C] and [4-^{3}H]ketone body tracers in rats.

We are aware of only one other stable isotope method for measurement of ketone body kinetics published

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TABLE 1.

Animal	Blood		Isotope Enrichment[a]			E_{AcAc}/E_{OHB}	Specific Activity[b]			SA_{AcAc}/SA_{OHB}	Turnover Rate	
	[AcAc]	[3-OHB]	E_{AcAc}	E_{OHB}	E_{KB}		SA_{AcAc}	SA_{OHB}	SA_{KB}		[3-^{14}C]	[$^2H_3$]
	mM		*atom % excess*			*%*	*dpm/µmol × 10^{-3}*			*%*	*µmol/min*	
1	0.70	1.46	2.37	2.66	2.56	89.0	14.70	18.06	16.97	81.4	17.9	19.8
2	0.58	2.30	2.06	3.15	2.93	65.4	12.08	15.18	14.56	79.6	20.8	17.9
3	0.97	2.47	1.60	2.42	2.18	66.1	9.37	12.99	11.97	72.1	25.4	24.2
4	0.57	2.41	3.15	4.06	3.89	77.6	23.59	28.00	27.15	84.3	11.2	13.3
5	0.63	2.07	3.15	3.81	3.66	82.6	22.77	28.83	27.42	79.0	11.1	14.2
Mean	0.69	2.14	2.47	3.22	3.04	76.1	16.50	20.61	19.61	79.3	17.3	17.9
SD	0.16	0.41	0.68	0.72	0.72	10.3	6.39	7.35	7.22	4.5	6.2	4.4

[a]E_{AcAc}, E_{OHB}, and E_{KB} are the [$^2H_3$] enrichments in circulating acetoacetate, 3-hydroxybutyrate, and total ketone bodies, respectively. The latter value is calculated as

$$E_{KB} = E_{AcAc} \times \frac{[AcAc]}{[KB]} + E_{OHB} \times \frac{[3OHB]}{[KB]}.$$

[b]SA_{AcAc}, SA_{OHB}, and SA_{KB} are the [3-^{14}C] specific activities of circulating acetoacetate, 3-hydroxybutyrate, and total ketone bodies, respectively. The latter value is calculated as

$$SA_{KB} = SA_{AcAc} \times \frac{[AcAc]}{[KB]} + SA_{OHB} \times \frac{[3OHB]}{[KB]}.$$

recently by Miles et al. (18). The present method differs from the latter technique as follows. *1*) Less sample is required in the current method. However, since Miles et al. (18) also employed ion monitoring GLC-MS analysis, it is likely that their sample size requirements could be reduced to match those reported here. *2*) Miles et al. (18) reported neither isotope detection nor precision measurements. However, these authors used a doubly ^{13}C-labeled ketone tracer and monitored the [M minus butyl] ion cluster of the *t*-butyldimethylsilyl (*t*-BDMS) derivative (m/z 273 and 275). Since this ionic species contains 12 carbon atoms, 2 oxygen atoms, and 2 silicon atoms, the "background" m+2/m ion current ratio due to natural abundance ^{13}C, ^{18}O, and ^{30}Si is approximately 8.5%. Thus, any [$^{13}C_2$]ketone body tracer enrichment must be measured above this natural "background." The present method, however, employs a triply labeled ketone tracer and the $[M-15]^+$ ion cluster of a trimethylsilyl derivative. The composition of this ion includes 9 carbons and 2 silicon atoms contributing a natural "background" m+3/m ion current ratio of only about 1.2% (3). Miles et al. (18) also reported in vitro loss of deuterons from synthesized [4,4,4-$^2H_3$]acetoacetate. This has not been our experience with 3-hydroxy[4,4,4-$^2H_3$]butyrate and Reed et al. (19) detected no loss of 3-hydroxy[4-^{3}H]butyrate after 18 months at −70°C.

It is also important to point out the complementary nature of the present method with that of Miles et al. (18). Simultaneous m+2/m and m+3/m ion monitoring is a routine GLC-MS technique. Thus, one could infuse both [3,4-$^{13}C_2$]acetoacetate tracer (18) and 3-hydroxy[4,4,4-$^2H_3$]butyrate tracer to determine simultaneously the kinetics of each ketone body and their rates of interconversion (22). This highly valuable approach has hitherto required separate tracer studies of [^{14}C]AcAc and [^{14}C]OHB on two different days (22). Using [$^{13}C_2$]AcAc and [$^2H_3$]OHB, however, individual ketone body kinetics could be measured simultaneously in the same study. Reed et al. (19) have successfully applied this principle to AcAc and 3-OHB kinetic measurements in rats with ^{14}C- and ^{3}H-labeled ketone bodies.

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REFERENCES

1. Hahn, P. 1978. Lipids. *In* Perinatal Physiology. U. Stave, editor. Plenum Publishing Corp., New York, NY. 397.
2. Hull, D. 1975. Storage and supply of fatty acids before and after birth. *Br. Med. Bull.* **31:** 32-36.
3. Robinson, A. M., and D. H. Williamson. 1980. Physiological roles of ketone bodies as substrates and signals in mammalian tissues. *Physiol. Rev.* **60:** 143-187.
4. Owen, O. E., A. P. Morgen, H. G. Kemp, J. M. Sullivan, M. G. Herrera, and G. F. Cahill, Jr. 1967. Brain metabolism during fasting. *J. Clin. Invest.* **46:** 1589-1595.
5. Kraus, H., S. Schlenker, and D. Schwedesky. 1974. Developmental changes of cerebral ketone body utilization in human infants. *Hoppe-Seyler's. Z. Physiol. Chem.* **355:** 164-170.
6. Persson, B., G. Settergren, and G. Dahlquist. 1972. Cerebral arteriovenous difference of acetoacetate and D-β-hydroxybutyrate in children. *Acta Paediatr. Scand.* **61:** 273-278.

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7. Adam, P. A. J., N. Räihä, E. L. Rahiala and M. Kekomaki. 1975. Oxidation of glucose and D-B-OH-butyrate by the early human fetal brain. *Acta Paediatr. Scand.* **64:** 17-24.
8. Settergren, G., B. S. Lindblad, and B. Persson. 1976. Cerebral blood flow and exchange of oxygen, glucose, ketone bodies, lactate, pyruvate and amino acids in infants. *Acta Paediatr. Scand.* **65:** 343-353.
9. Bougneres, P. F., I. E. Karl, L. S. Hillman, and D. M. Bier. 1982. 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.
10. Melichar, V., V. Drahota, and P. Hahn. 1967. Ketone bodies in the blood of full term newborns, premature and dysmature infants and infants of diabetic mothers. *Biol. Neonate.* **11:** 23-28.
11. Persson, B., and J. Gentz. 1976. The pattern of blood lipids and ketone bodies during the neonatal period, infancy and childhood. *Acta Paediatr. Scand.* **55:** 353-362.
12. Anday, E. K., C. A. Stanley, L. Baker, and M. Delivoria-Papadapoulos. 1981. Plasma ketones in newborn infants: absence of suckling ketosis. *J. Pediatr.* **98:** 628-630.
13. Williamson, D. H., and J. Mellanby. 1974. D-(−)-3-hydroxybutyrate. *In* Methods of Enzymatic Analysis. Volume 4. H. U. Bergmeyer, editor. Academic Press, New York, NY. 1836-1837.
14. Diagnosis of Organic Acidemias by Gas Chromatography-Mass Spectrometry. 1981. S. I. Goodman and S. P. Markey, editors. Alan R. Liss, New York, NY. 128 and 131.
15. Mass Spectra of Compounds of Biological Interest. Volume II, Part 1. U.S. Atomic Energy Commission, publication TID-26553-P2. 292 and 298.
16. Balasse, E. O. 1979. Kinetics of ketone body metabolism in fasting humans. *Metabolism.* **28:** 41-50.
17. Balasse, E. O., and R. J. Havel. 1971. Evidence for an effect of insulin on the peripheral utilization of ketone bodies in dogs. *J. Clin. Invest.* **50:** 801-813.
18. Miles, J. M., W. F. Schwenk, K. L. McClean, and M. W. Haymond. 1984. Determination of ketone body turnover in vivo with stable isotopes, utilizing gas chromatography/mass spectrometry. *Anal. Biochem.* **141:** 110-115.
19. Reed, W. D., P. J. Baab, R. L. Hawkins, and P. T. Ozand. 1983. A double-isotope method for the measurement of ketone-body turnover in the rat. *Biochem. J.* **219:** 15-24.
20. Bates, M. W., H. A. Krebs, and D. H. Williamson. 1968. Turnover rates of ketone bodies in normal, starved and alloxan-diabetic rats. *Biochem. J.* **110:** 655-661.
21. Keller, U., G. E. Sonnenberg, and W. Stauffacher. 1981. Validation of a tracer technique to determine nonsteady-state ketone body turnover rates in man. *Am. J. Physiol.* **240:** E253-E262.
22. Cobelli, C., R. Nosadini, G. Toffolo, A. McCulloch, A. Avogaro, A. Tiengo, and K. G. M. M. Alberti. 1982. Model of the kinetics of ketone bodies in humans. *Am. J. Physiol.* **243:** R7-17.

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