Ketone body kinetics in humans: a mathematical model

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Abstract A model has been developed to account for ketone body kinetics in man based on data following bolus injections of [^{14}C]acetoacetate (A) and [^{14}C]β-OH butyrate (B) into normal humans in the postabsorptive state. The model consists of separate compartments for blood A and B that are linked by a tissue compartment in which rapid interconversion of the ketone bodies occurs. The probability of movement from blood into this compartment was assumed to be the same for both ketone bodies. Two slowly equilibrating tissue compartments are required to account for the slow components in the tracer data, and thus a five-compartment model is proposed. By modeling the transient tracer data with the tracee in a steady state, ketone body kinetics were defined in terms of the rapid interconversions of A and B, and the slow exchanges of carbon within the tissues. The rates of release of new A and B into blood, (U_A and U_B) were calculated. These rates were less than the apparent production rates, PR_A and PR_B, as the PR's included carbon atoms first released as the other ketone body. The exchange constants between the compartments were determined in addition to the fractional catabolic rates (FCR) and metabolic clearance rates (MCR) of A and B. The initial space of distribution was 10 L and the mean values ± SD (n = 11), normalized to this volume, were $U_A = 6.4 \pm 5.0$, $U_B = 8.8 \pm 8.0$ (μmol L^{-1} min^{-1}), $FCR_A = 0.226 \pm 0.142$, $FCR_B = 0.188 \pm 0.124$ (min^{-1}), $MCR_A = 2.26 \pm 1.42$, $MCR_B = 1.87 \pm 1.23$ (L min^{-1}) and $PR_A = 11.1 \pm 7.6$, $PR_B = 12.7 \pm 10.0$ (μmol L^{-1} min^{-1}).—**Wastney, M. E., S. E. H. Hall, and M. Berman.** Ketone body kinetics in humans: a mathematical model. *J. Lipid Res.* 1984. **25:** 160–174.

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The ketone bodies acetoacetate (A), β-OH butyrate (B), and acetone (Ac) are normally present at low concentrations in the blood (<0.2 mM) and are considered to have a minor role in metabolism. However, when glycogen reserves are depleted and free fatty acid concentrations increase, such as in starvation and diabetes, concentrations can increase 50-fold to >10 mM. In these situations ketone bodies are considered to be important oxidative substrates (1).

While the biochemistry of ketone body metabolism has been studied in detail (for reviews see 2–4), the kinetics of metabolism in vivo have not been fully quantified. Experiments employing ^{14}C-labeled A or B have been undertaken in man (5–11), dogs (12–14), and rats (15–21). However, tracer experiments have been beset by several problems which make interpretation of the data difficult. Until recently (8) none of the approaches permitted separate, simultaneous studies of the two major ketone bodies in blood, A and B, despite the fact that under many physiological conditions the concentrations of the two keto acids in blood do not change in parallel. In fact, valuable information is lost when the two are treated as a single entity.

In an attempt to elucidate ketone body metabolism in man, experiments with [3-^{14}C]acetoacetate and [3-^{14}C]β-hydroxybutyrate were undertaken in normal subjects and the data were used to develop a model of ketone body metabolism. The kinetics of A and B in blood were defined separately, as this is important in determining the regulation of ketone body metabolism.

METHODS

Subjects and experimental design

All subjects gave their informed written consent to the experiments. They were healthy, of normal weight to height^2 ratio (22), and had no family history of diabetes. Experiments were carried out while the subjects were warm, relaxed, and semi-supine in bed. Most slept through the experiment.

The tracers used were [^{3}-^{14}C]acetoacetate (A*) and D-[3-^{14}C]β-hydroxybutyric acid (B*) (Amersham Corp.). A* was injected into an antecubital vein as a single bolus in three subjects (KN14, KN15, and KN16) in the postabsorptive state. The same individuals were given a bolus injection of B* by the same protocol about a week later.

Abbreviations: A, acetoacetate; B, β-OH butyrate; Ac, acetone.

[1] Dr. Berman died while this manuscript was in preparation.

The experiments were alternated. Eight additional subjects received A* by continuous intravenous infusion over 4 hr.

Blood samples were taken at 1, 2, 4, 7, 9, 15, 20, 35, 50, 60, 80, 120, 150, and 180 min following the bolus injection of tracer and 20, 40, 60, 80, 100, 120, 140, 160, 200, and 240 min after the start of the infusion experiments, from a contralateral antecubital vein via a polythene cannula fitted with a three-way tap.

Pertinent details on the subjects (n = 11) are shown in **Table 1.**

Preparation of tracers

A*, [^{14}C-3]Na-A, was prepared from [^{14}C-3]ethyl acetoacetate (5). As found by others (23), 86 ± 4% of ethyl acetoacetate was converted to A. Aliquots of 1.5 ml of 0.05 M Na-A in 0.9% saline was stored at −20°C. Batches were stored for not longer than 1 week. On the day of the study, the tracer was diluted to a final volume of 50 ml for infusion, or injected directly in 1.5 ml containing approximately 100 μCi of A* or B*. Tracer solutions were sterilized by Millipore filtration before injection or infusion.

Chemical analysis

The concentrations of A and B were measured enzymically (24) in blood samples that had been deproteinized with perchloric acid.

To isolate ^{14}C-labeled A and B, blood samples (5 ml) were plunged immediately into preweighed tubes containing 5 ml of ice-cold perchloric acid. The exact volume of blood was determined by weight difference.

Samples from experiments where the tracer was injected were lyophilized to remove all ^{14}C-labeled acetone (Ac*). In seven of the infusion experiments, Ac* was removed by bubbling nitrogen through the samples for 30 min (25). However, adding ^{14}C-labeled Ac to blood filtrates showed that acetone was only partially (53.0 ± 5%) removed by this procedure.

The reconstituted freeze-dried extract or deproteinized sample was divided into two equal aliquots for the isolation of ^{14}C-labeled A and ^{14}C-labeled B as the Denigès salt, with B first being converted to A (19). An aliquot of the tracer solution was mixed with blood, taken prior to the experiment, and processed with the samples for estimation of the dpm injected or infused. Nearly all Na-acetate formed in the conversion of ethyl acetoacetate to sodium acetoacetate (13) was removed and only approximately 0.2% was converted to the Denigès salt (26). The recoveries of the ketone bodies as the Denigès salt were complete.

TABLE 1. Details of subjects

Subject	Sex	Age	Weight	BSA[a]
		yr	*kg*	m^2
KN03	M	25	76.8	1.95
KN04	M	44	80.0	2.01
KN05	M	35	71.7	1.80
KN06	M	40	78.4	2.07
KN07	M	23	70.8	1.87
KN08	M	35	49.7	1.53
KN09	F	56	66.6	1.70
KN10	M	51	76.3	1.93
KN14	F	56	57.4	1.59
KN15	M	27	70.8	1.86
KN16	M	62	88.3	2.09
Mean		41	71.5	1.86
SD		14	10.7	0.19

[a] Body surface area, calculated from the formula of DuBois (see Ref. 48).

Data analysis

The data were analyzed by compartmental analysis (27–30) using the SAAM (31) and CONSAM (32) programs on a VAX 11/780 computer (Digital Equipment Corp., Marlboro, MA).

MODEL DEVELOPMENT

Model for A and B

The model was developed from data of tracer injection studies (A* and B*) in the same individual (**Fig. 1**). The data from each subject were fitted separately and the nomenclature used is explained on **Table 2.** The model was then used to fit the studies of constant tracer infusions of A* (**Fig. 2**).

In developing the model, some features were introduced on the basis of known physiology and biochemistry while others were dictated by the data. The initial space of distribution of A and B was calculated from the intercepts of the decay curves (fraction of dose ml^{-1} vs time) following the bolus injections of tracer, and the mean value was used in the injection studies. A and B were found to have the same space of about 10 L or 14% of body weight.

From biochemical studies (see 24) we know that ketone bodies are produced primarily by the liver, although some may be produced de novo by other tissues during exercise or starvation (33, 34). All tissues with the possible exception of the liver can utilize A and B for energy. In addition, all tissues, but not blood, possess β-hydroxybutyrate dehydrogenase and can therefore convert A to B and vice versa depending on the prevailing NAD:NADH ratios (4). With this in mind, we initially attempted to fit a six-compartment model as shown in **Fig. 3a.**

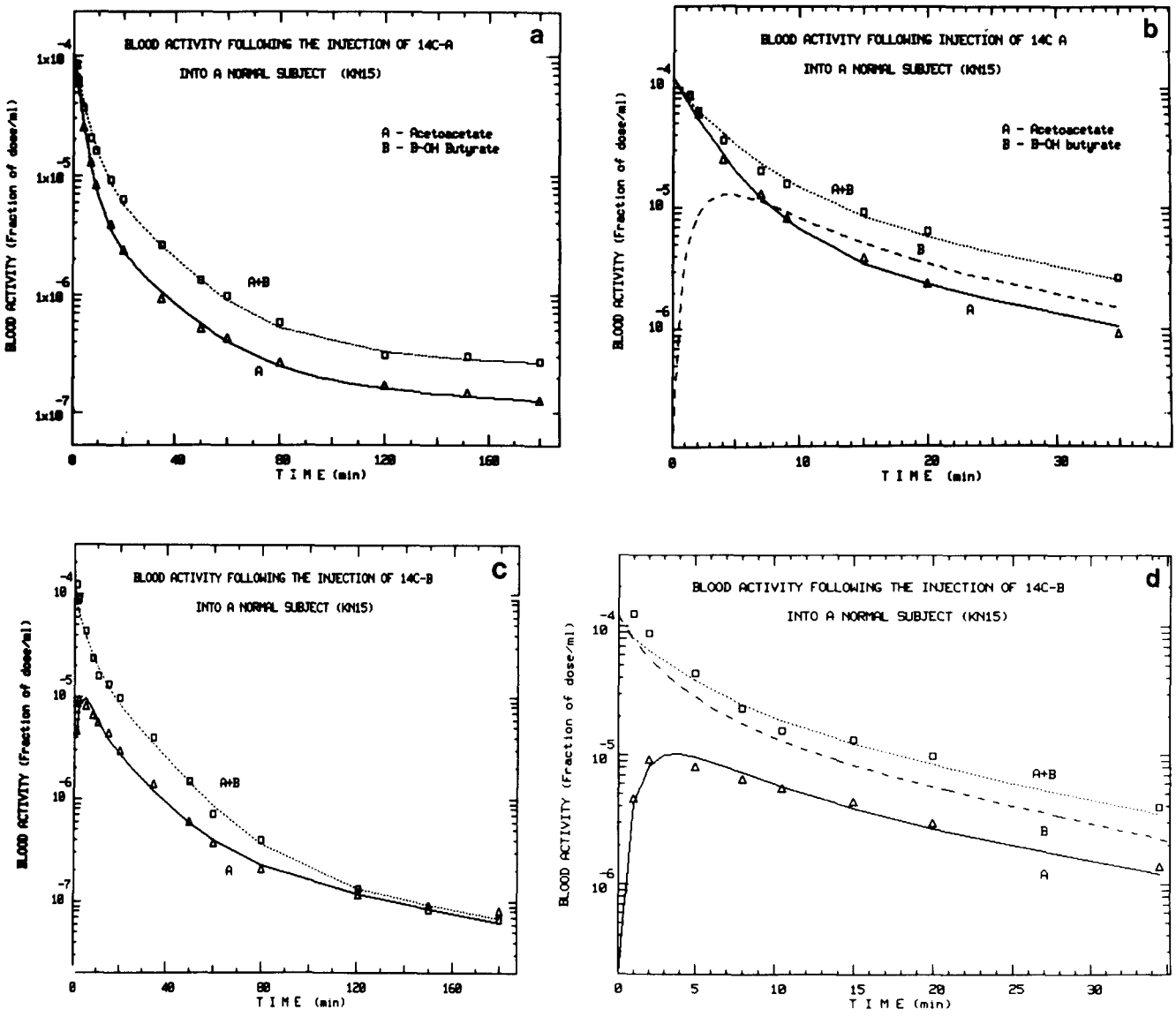

Fig. 1. The model-generated curves and observed data of blood tracer disappearance, following a, the injection of A* into a subject in the postabsorptive state and b, the early portion of the curves showing the rapid appearance of label in blood B. c, The curves following an injection of B* and d, the early portion of the curves following the injection of B*. The data are expressed as measured, i.e., as A* + B* and A*. B* was calculated by difference.

This model included separate compartments for A and B in blood, liver, and tissues. However, as label from one ketone body appeared in the other within 2 min (Figs. 1b, 1d), we concluded, as did others (8, 15, 17), that A and B could not be distinguished once they had entered the peripheral compartments and they were treated as single entities in compartments 2 and 6 (Fig. 3b). Another slowly turning over pool (compartment 3) was required to fit the final slope of the tracer disappearance curves (Fig. 1). This compartment probably contains compounds that exchange carbon with ketone bodies. These would include fatty acids, amino acids, and rapidly turning over proteins (12).

Physiologically, the major site of ketone body production is the liver (21) and utilization occurs in other tissues. In this model (Fig. 3b) fast interconversion occurred through compartment 2 (possibly liver) while slower interconversion occurred through compartment 6 (possibly tissues). Considering that the liver receives only 25% of the cardiac output, the rate coefficient representing entry into the liver ($L_{2,1}$) could not exceed 0.25 min^{-1}. However, it generally had a value larger than this (**Appendix 1** shows the results for one study). On this basis we could not justify that compartment 2 was solely liver and we reasoned that some fast interconversion was also occurring in the tissues.

TABLE 2. Nomenclature

Parameter	Name	Definition
FCR_i	Fractional catabolic rate	Fraction of material in compartment i which leaves irreversibly, per unit time
$L_{i,j}$	Rate constant	Fractional flow into compartment i from j
$LI_{i,j}$	L-inverse	Mass generated in compartment i by a unit infusion into compartment j
$LN_{i,j}$	Normalized L-inverse	Fraction of compartment j which passes through compartment i
MCR_i	Metabolic clearance rate	Volume of blood cleared per unit time
T_i	Residence time	Mean time that a particle spends in compartment i
Tracee:		
$a_{i,j}$	Specific activity	Ratio of tracer to tracee in compartment i, when tracer enters compartment j
C_i	Concentration	Mass per unit volume
M_i	Mass	Tracee in compartment i
PR_i	Production rate	Rate of entry of new particles into compartment i for the first time
$R_{i,j}$	Flow rate	Tracee flow into compartment i from compartment j
U_i	Synthesis	Entry of tracee into the system
U_A		Appearance of A in blood for the first time, as A
Tracer:		
$c_{i,i}$ (t)	Tracer concentration	Activity per unit volume in compartment i
$f_{i,j}$ (t)		Tracer observed in compartment i with an input into compartment j
u_i (t)		Tracer input into compartment i
u_c		Tracer infusion into compartment i
V_i	Volume	Initial volume of distribution

An attempt was made to divide compartment 2 into two pools, representing liver and extrahepatic tissues (compartments 2 and 9 in Fig. 3c). Although a good fit to the data was obtained with this model, some of the parameters of the system were not well determined (see Appendix 1). It was concluded that from blood tracer data it was not possible to resolve separate compartments for the liver and tissues, respectively.

The model was condensed to the final form used for data analysis (Fig. 3d). In this model all fast interconversion occurred through a combined tissue and liver compartment and degradation of ketone bodies ($L_{0,2}$) was assigned to this compartment, since none takes place directly in blood. This was linked in series to two slowly turning over compartments, 6 and 3. These three compartments represent compounds that exchange carbon with ketone bodies. The sites of interconversion are extravascular and are distributed throughout the liver and tissues.

Adjustment of the parameters $L_{2,1}$, $L_{1,2}$, $L_{4,2}$, $L_{2,4}$, and $L_{0,2}$ permitted the fitting of the initial transient curves for both blood A and B. Based on their biochemical properties, and to minimize the number of parameters in the model, we assumed that A and B molecules in blood had the same probability of moving from blood into the tissues and the fractional transfer rates were set to be equal, ($L_{2,1} = L_{2,4}$). The fractional clearance rates of A and B, (FCR_A, FCR_B) from blood, however, were not affected by this as $L_{1,2}$ was not equal to $L_{4,2}$ (see equation 4). In addition, we assumed that the ratio of A to B in tissues was the same as in blood, and that tracer was returned to blood from tissues in this ratio. Thus $L_{1,2}/(L_{1,2} + L_{4,2})$ was equated to the ratio of blood A to blood B.

In several of the experiments where the tracer was injected, the activity curves of A* and A* + B* converged (Fig. 1c). This may have been due to a substance that was injected with the ketone bodies but eliminated from the blood at a slower rate. Only a negligible fraction (0.7%) of the injected counts would account for this final slope.

The infusion experiments were fitted to the same model and the parameters were weighted by the mean values obtained from the bolus injection studies. The volumes of distribution were fixed at 10 L. In addition, as samples from our infusion studies contained some Ac*, we included a small pathway to represent the formation of Ac.

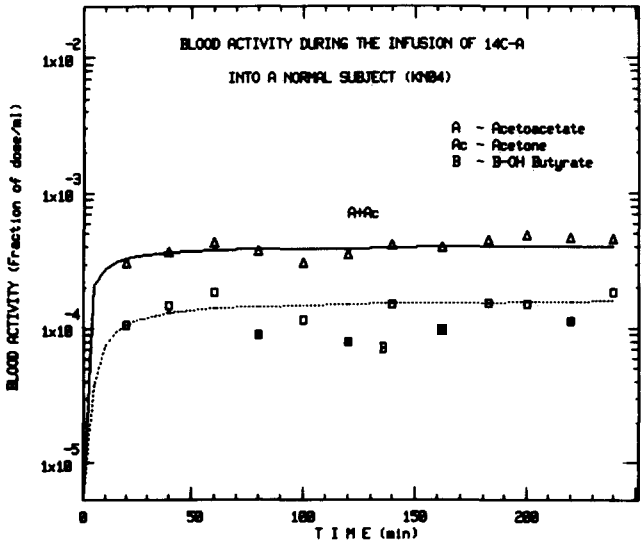

Fig. 2. The model-generated curves and observed radioactivity on A and B during a 4-hr infusion of A* into a normal subject in the postabsorptive state. The solid points were given zero weight in the data fitting.

In most published tracer studies, the presence of Ac* as a contaminant of A* in blood samples has been generally disregarded and often no attempt has been made to remove it, although Reichard et al. (35) showed that its inclusion can lead to erroneous estimates of the concentration of A* in the blood. Furthermore, as Ac turns over very slowly (35), the presence of Ac* would influence the final slope of the A* curve. It is known to be formed spontaneously from A and by synthesis by some tissues (13). Since the relative contributions of these pathways is not known, one pathway, representing <1% of the loss from compartment 2, was included. The turnover rate of Ac was fixed at 0.003 min^{-1} (35) while the concentration was calculated as 0.15 that of blood A (9). The space of distribution was fixed at 75% of bodyweight (35). Thus the mass and the rate of loss could be determined, and hence the rate of formation,

Rate of formation of acetone

$$= \text{rate of loss} = \text{mass} \times 0.003\ min^{-1}. \quad Eq.\ 1)$$

Using these parameters, some acetone was included in the analyses of the studies where it was not completely removed.

The blood tracee concentrations occasionally drifted during the experiments, however the ratio of blood A to B was generally maintained and the system was treated as time invariant over the duration of the study period.

Input of newly synthesized ketone bodies was assigned to compartment 2, the rapidly mixing tissue compartment, as U_2. It was assumed that the ratio of A:B in the blood reflected the ratio that A and B were returned to the

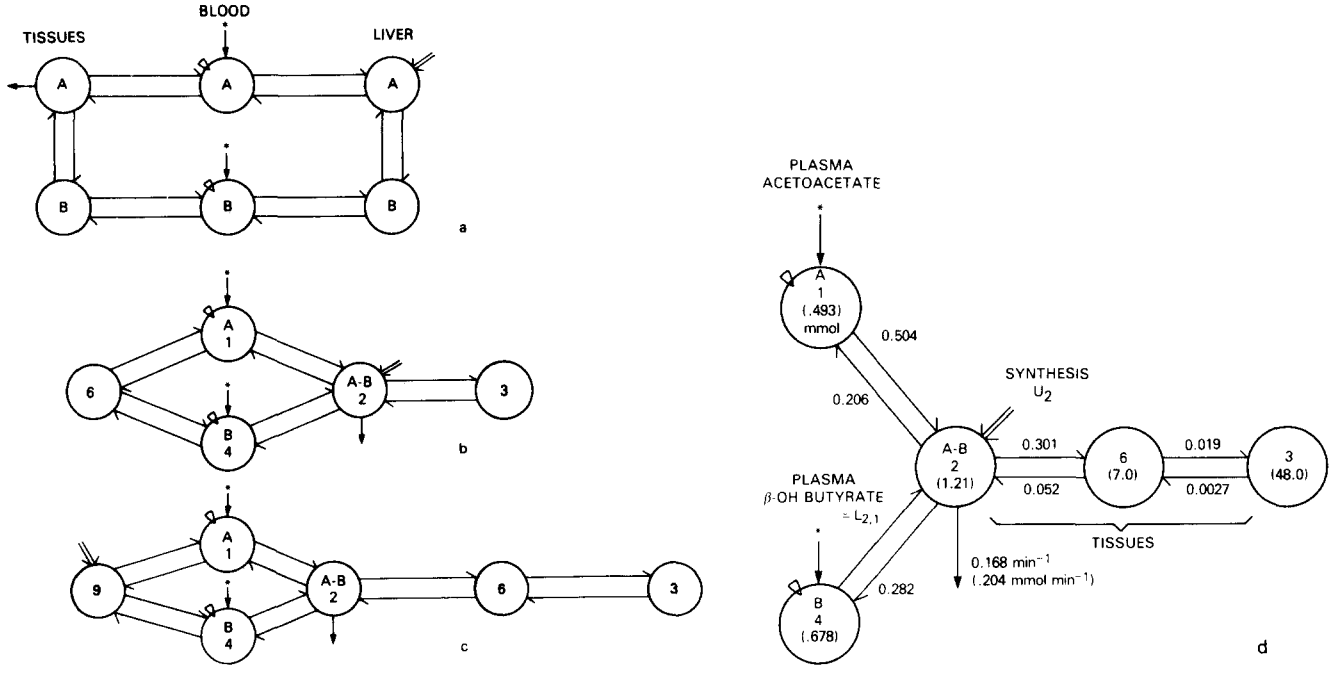

Fig. 3. a, A six-compartment model, based on known biochemistry, initially proposed to explain ketone body kinetics. The starred arrows represent tracer entry sites and the triangles mark pools that were sampled. b, A reduced model with the A and B in the liver and tissues represented by single compartments. c, A model with blood A and B linked through two fast compartments. d, The five-compartment model proposed for ketone body kinetics in man. Compartments 1 and 4 represent blood acetoacetate (A) and β-OH butyrate (B), respectively, while compartments 2, 6, and 3 are extravascular pools that exchange carbon with ketone bodies. The average values for the rate coefficients (min^{-1}), masses (mmol), and utilization rate of ketone bodies (mmol min^{-1}) are shown.

blood by extrahepatic tissues. This was consistent with the data for most studies. In some an additional input of tracee, as A, U_1, or B, U_4, was also required. This implied that new material was entering blood in a ratio different from material being returned to blood from tissues. This is a consequence of the tracer entering the system at a site (blood) different from the newly synthesized material, or lack of equivalence of tracer and tracee supply (36).

Model-based calculations

Some parameters of the ketone body system, such as production rate (PR), fractional catabolic rate (FCR), and clearance (MCR), can be calculated directly from the areas under the blood activity vs time curves (27, 28). However, once a model has been developed, these and other parameters of the system can be determined from the calculated parameters of the model and the steady state tracee measurements (27, 28). To describe the model-based calculations it is most convenient to use a matrix representation for the rate constants, $L_{i,j}$, and apply some simple matrix transformations, such as the calculation of a matrix inverse to derive the desired expressions.

If L is the matrix of the rate constants $L_{i,j}$ (i not equal to j, and $L_{i,j} > 0$) and the inverse of the L matrix is defined as LI, then the element of the inverse matrix, $LI_{i,j}$, is the expected residence time in compartment i of particles entering compartment j (28). $LI_{i,j}$ is also the steady state mass in compartment i generated by a unit infusion rate ($U_j = 1$) into compartment j (28), and equals the area under a tracer curve in compartment i after a unit bolus injection into compartment j (28).

The normalized inverse, $LN_{i,j}$, is equivalent to division of each row of the inverse matrix by its diagonal element,

$$LN_{i,j} = LI_{i,j}/LI_{i,i} \qquad Eq.\ 2)$$

and represents the fraction of material in compartment j which will reach compartment i. These and other methods of calculation are given in **Table 3,** using the notation in Table 2.

Transport rate (or total entry rate) has been defined as the rate that tracee moves into (or out of) a compartment (37). For A in this model it is ($U_1 + R_{1,2}$) or $R_{2,1}$. Production rate (or irreversible disposal rate) is the mass of newly synthesized A which is entering the compartment for the first time (38), called PR_A. This rate however is comprised of new molecules that have never been in blood (called U_A) plus some fraction (LN_{AB}) of new molecules that first entered blood as B (U_B) and were then converted to A.

The PR's can be expressed as:

$$PR_A = U_A + LN_{AB} \cdot U_B \qquad Eq.\ 3a)$$

$$PR_B = U_B + LN_{BA} \cdot U_A. \qquad Eq.\ 3b)$$

U_A and U_B therefore represent the arrival of newly synthesized ketone bodies in blood as A or B, respectively. Given the fraction of blood A converted to blood B (LN_{BA}) and B converted to A (LN_{AB}), U_A and U_B can be calculated from the values of PR_A and PR_B, by the equations shown in Table 3. The equation for $U_A + U_B$ is equivalent to that derived by Barton (39) and Heath and Barton (40) for what they define as $v_1 + v_4$.

One measure of utilization is FCR. The parameter is model-independent (28), if the tracer and the tracee behave in the same manner. This was a basic assumption in our studies. For the model presented these are:

$$FCR_A = 1/LI_{1,1} = L_{2,1} \cdot L_{0,2}/(L_{0,2} + L_{1,2}) \qquad Eq.\ 4a)$$

$$FCR_B = 1/LI_{4,4} = L_{2,4} \cdot L_{0,2}/(L_{0,2} + L_{4,2}). \qquad Eq.\ 4b)$$

RESULTS

Individual and population values of the transfer coefficients are given in **Table 4.** The error estimate of the individual parameters was determined by SAAM (31) while the population errors were determined by weighting the individual values by the inverse of their standard deviations (41), as described on Table 4.

The synthesis and production rates of A and B are given in **Table 5** and the blood concentrations, interconversions, and clearance rates of A and B are given in **Table 6.** The mean parameter values are compared to values in the literature in **Table 7.** Relatively few studies are available on normal humans in the postabsorptive state.

The ketone bodies in blood have turnover times of about 2 min (Table 4) and this is consistent with rapid movement out of blood. They move into a mixing pool (compartment 2) that probably represents ketone bodies within the tissues and liver. This compartment turns over in about a minute and has a space of 21 L, or 30% of body weight, which is comparable to total body intracellular fluid (40% of body weight).

The results for the normal population suggest that newly produced ketone bodies are released into blood as A or B in comparable amounts (U_A = 6.4 vs U_B = 8.8 μmol L^{-1} min^{-1}, Table 5), while the corresponding production rates PR_A and PR_B (11.1 and 12.7 μmol L^{-1} min^{-1}, Table 5), are 73% and 44% higher, respectively. The fractional interconversion of A and B (derived directly from the rate constants of the model as described earlier, Table 6), show that 62% of blood A is converted to blood B while 54% of blood B is converted to blood A.

Most of the variation between individuals was in the rate coefficients $L_{2,1}$, $L_{1,2}$, and $L_{4,2}$, although the studies of KN08, KN09, and KN10 had lower values for $L_{0,2}$

TABLE 3. Expressions for some kinetic properties of a system

Symbol	Comp. Model	Tracer Data: Injection	Tracer Data: Infusion	Tracee
Residence time				
T_i	$LI_{i,i}$	$\frac{\int_0^\infty f_{i,i}(t)dt}{\int_0^\infty u_i(t)dt}$	$\frac{f_{i,i}(\infty)}{u_c}$	$\frac{M_i}{PR_i}$
Fractional catabolic rate				
FCR_i	$\frac{1}{LI_{i,i}}$	$\frac{\int_0^\infty u_i(t)dt}{\int_0^\infty f_{i,i}(t)dt}$	$\frac{u_c}{f_{i,i}(\infty)}$	$\frac{PR_i}{M_i}$
Production rate				
PR_i	$\frac{M_i}{LI_{i,i}}$	$\frac{\int_0^\infty u_i(t)dt}{\int_0^\infty a_{i,i}(t)dt}$	$\frac{u_c}{a_{i,i}(\infty)}$	$M_i \cdot FCR_i$
	$U_i + \sum LN_{i,j}U_j$			
Transfer coefficient				
$LN_{i,j}$	$\frac{LI_{i,j}}{LI_{i,i}}$	$\frac{\int_0^\infty a_{i,j}(t)dt}{\int_0^\infty a_{i,i}(t)dt}$	$\frac{a_{i,j}(\infty)}{a_{i,i}(\infty)}$	
Input rate				
U_i	$\sum L_{i,j}M_j$			
Metabolic clearance rate				
MCR_i	$\frac{V_i}{LI_{i,i}}$	$\frac{\int_0^\infty u_i(t)dt}{\int_0^\infty c_{i,i}(t)dt}$	$\frac{u_c}{c_{i,i}(\infty)}$	$\frac{PR_i}{C_i}$
Synthesis rate				
U_A	$\frac{PR_A - LN_{AB}PR_B}{1 - LN_{AB}LN_{BA}}$			
U_B	$\frac{PR_B - LN_{BA}PR_A}{1 - LN_{AB}LN_{BA}}$			
$U_A + U_B$	$\frac{(1 - LN_{BA})PR_A + (1 - LN_{AB})PR_B}{1 - LN_{AB}LN_{BA}}$			

(Table 4). The concentrations of A and B in these subjects were almost double those observed in the other studies (Table 6). In studies KN09 and KN10, ketogenesis was increased ($U_A + U_B$, Table 5) whereas the increased concentrations in KN08 were fully explained by the decrease in the rate constant for utilization ($L_{0,2}$). One study (KN16B, Table 6) differed from most of the others in that the blood A concentration was greater than B, and this was inconsistent with predictions from the tracer kinetics.

The utilization rate includes oxidation and urine loss. Since the ratio of A:B in compartment 2 equals $L_{1,2}/L_{4,2}$, these losses may be expressed in terms of A only by dividing the rate constant $L_{0,2}$ by the fraction $L_{1,2}/(L_{1,2} + L_{4,2})$.

Reproducibility

The reproducibility of the studies was tested by carrying out duplicate studies on three subjects (KN14, KN15, and KN16) 1 week apart. In each case there were changes

TABLE 4. Rate constants of ketone body kinetics in normal subjects

Study	$L_{1,2}$	$L_{4,2}$	$L_{2,1}$	$L_{0,2}$	$L_{6,2}$	$L_{2,6}$	$L_{3,6}$	$L_{6,3}$
				min^{-1}				
KN03	0.140	0.236	0.329	0.312	0.210	0.069	0.031	2.53E-3
			0.024	0.078	0.096	0.027	0.010	1.18E-3
KN04	0.449	0.289	0.632	0.220	0.291	0.063	0.034	3.37E-3
			0.118	0.063	0.095	0.026	0.010	1.16E-3
KN05	0.090	0.145	0.356	0.262	0.288	0.065	0.029	2.91E-3
			0.021	0.081	0.101	0.026	0.010	1.13E-3
KN06	0.340	0.120	0.510	0.202	0.327	0.058	0.022	3.17E-3
			0.105	0.067	0.094	0.025	0.009	1.09E-3
KN07	0.261	0.312	0.484	0.138	0.337	0.064	0.024	3.39E-3
			0.064	0.044	0.080	0.021	0.008	0.96E-3
KN08	0.020	0.049	0.174	0.006	0.364	0.047	0.00	3.18E-3
			0.008	0.014	0.107	0.020		1.20E-3
KN09	0.080	0.208	0.894	0.058	0.295	0.027	0.004	3.04E-3
			0.070	0.013	0.081	0.011	0.004	1.20E-3
KN10	0.316	0.485	0.883	0.053	0.377	0.059	0.014	3.23E-3
			0.078	0.018	0.086	0.017	0.006	1.15E-3
KN14	0.205	0.549	0.340	0.307	0.146	0.063	0.035	2.73E-3
			0.026	0.061	0.066	0.024	0.010	1.15E-3
KN14B[a]	0.079	0.491	0.597	0.128	0.095	0.077	0.033	3.60E-3
			0.053	0.024	0.038	0.024	0.008	1.08E-3
KN15	0.166	0.258	0.458	0.209	0.364	0.081	0.035	3.48E-3
			0.031	0.053	0.086	0.019	0.006	0.89E-3
KN15B	0.275	0.629	0.582	0.367	0.274	0.143	0.001	3.00E-3
			0.049	0.058	0.068	0.018	0.001	0.17E-3
KN16	0.214	0.303	0.394	0.264	0.424	0.068	0.029	0.89E-3
			0.032	0.067	0.096	0.018	0.007	0.65E-3
KN16B[b]	0.320	0.846	0.723	0.201	0.242	0.015	0.031	3.39E-3
			0.075	0.067	0.072	0.005	0.006	0.73E-3
Mean	0.206	0.282	0.504	0.168	0.301	0.052	0.019	2.78E-3
SD	0.128	0.152	0.222	0.109	0.086	0.019	0.012	0.88E-3

Value ± SD. The population means were calculated from the individual values (and average of the duplicate experiments) weighted in inverse proportion to $(S_i^2 + S_p^2)$ where S_i is the SD of the individual and S_p is the SD of the population (41).

[a] B refers to a bolus injection of B*. In all the other studies, the tracer was A*.

[b] The second study on KN16 was not included in the calculations (see text).

in the blood concentrations of A and B (Table 6) and in several of the model rate coefficients, namely $L_{1,2}$, $L_{4,2}$, and $L_{0,2}$. Some of the parameters changed by more than 100% (Table 4). Since ketone body concentrations change from day to day, experiments where A and B are injected simultaneously, with different labels, are necessary to resolve this problem.

DISCUSSION

A model for ketone body kinetics, which accounts for observed transient data and tracee concentrations under various experimental protocols and physiological states, is presented together with parameter values for normal subjects. These serve as a reference against which metabolism of perturbed states may be compared.

Several models have been proposed for ketone body kinetics (8, 17–19, 21). Bates, Krebs, and Williamson (19) and McGarry, Guest, and Foster (21) used a single-pool model for analyzing ketone body kinetics in rats. They assumed that ketone bodies were quickly interconverted and distributed throughout the body. This model was later expanded by Bates (17, 18) to three pools, as the single-pool model could not explain the disequilibrium of the specific activities of A and B during the infusion

TABLE 5. Initial volume of distribution, synthesis, and production rates of ketone bodies in normal subjects

Study	Vol	Synthesis Rate U_1	Synthesis Rate U_4	Synthesis Rate U_2	Production Rate PR_A	Production Rate PR_B	Blood Appearance U_A	Blood Appearance U_B	Blood Appearance $U_A + U_B$
	L				*μmol L⁻¹ min⁻¹*				
KN03	10.0			26.8	8.3	11.5	5.4	9.1	14.6
				6.0	1.0	2.0	0.8	1.9	2.4
KN04	10.0			17.4	11.7	9.9	8.2	5.2	13.4
				4.1	1.9	1.6	1.4	1.3	2.4
KN05	10.0			39.4	10.1	14.0	7.1	11.5	18.6
				7.6	1.1	1.8	0.8	1.7	2.4
KN06	10.0			10.4	6.5	3.9	5.3	1.9	7.2
				3.3	1.4	0.8	1.2	0.6	1.8
KN07	10.0			8.4	5.4	5.7	3.0	3.7	6.7
				2.0	0.9	1.0	0.6	0.8	1.3
KN08	10.0			5.1	3.0	3.6	1.3	3.3	4.6
				1.0	5.3	7.3	2.2	5.5	8.0
KN09	10.0			46.6	26.0	35.3	10.7	27.9	38.6
				14.8	7.8	11.0	3.3	8.9	12.1
KN10	10.0		36.0		29.9	35.1	0.0	36.5	36.5
			14.1		10.5	14.0		14.1	14.8
KN14	10.0	12.6		10.0	16.2	14.0	14.5	5.1	19.6
		9.0		11.8	5.0	3.0	7.0	6.0	3.7
KN14B	10.0	24.5			24.2	19.2	24.5	0.0	24.5
		8.8			8.7	7.0	8.8		8.8
KN15	8.3	5.7		11.5	10.7	9.3	8.7	4.7	13.4
		5.6		12.0	1.3	3.5	2.7	4.9	3.0
KN15B	8.3	9.3		20.6	17.9	18.6	13.7	10.1	23.8
		4.1		7.0	1.8	2.0	2.7	3.2	1.8
KN16	12.1	9.1		20.5	18.2	15.7	14.7	7.9	22.6
		9.7		20.1	3.0	5.6	4.9	7.6	5.3
KN16B	12.1	17.9			17.8	14.4	17.9	0.0	17.9
		6.3			6.3	4.7	6.3		6.3
Mean				20.6	11.1	12.7	6.4	8.8	15.2
SD				19.7	7.6	10.0	5.0	8.0	12.3

Value ± SD. Population values were generated by the model when run with the mean population rate constants. Values are normalized to the initial volume of distribution.

[a] The initial volume of distribution was determined from the injection studies (KN14, KN15, and KN16) and fixed at 10 L for the infusion studies.

of one tracer. The three-pool model had separate pools for blood A and B and assumed that interconversion of ketone bodies occurred in a single compartment, the liver. This was based on the work of McGarry et al. (21) where no interconversion of A and B was observed in hepatectomized rats.

Cobelli et al. (8) tested the three-compartment model on ketone body data in man, but found that it was inadequate to fit all data. Consequently they proposed a four-compartment model (8), with rapid interconversion occurring in a tissue compartment and slow interconversion in a liver compartment. However, it is not clear whether the slow compartment is solely liver as it has a mass that is nine times the size of plasma A or B, and six times the size of the tissue pool. This suggests that the liver is a storage area for ketone bodies and differs from an observation in rats (42) where the concentration of ketone bodies was one-half that of plasma. Based on the relative volume of liver to plasma, one would expect a much smaller pool of compounds containing ketone body carbon in liver. In addition, as discussed in the model development section, the fractional uptake by the com-

TABLE 6. Blood concentrations, interconversion, and clearance rates of ketone bodies in normal subjects

	Conc.		Interconversion		Clearance			
	A	B	LN_{BA}	LN_{AB}	FCR_A	FCR_B	MCR_A	MCR_B
	$\mu mol\ L^{-1}$				min^{-1}		$L\ min^{-1}$	
KN03	36.4	61.0	0.43	0.31	0.226	0.187	2.260	1.871
	4.4	12.0	0.33	0.03	0.010	0.011	0.106	0.118
KN04	56.0	36.0	0.57	0.67	0.207	0.273	2.075	2.726
	6.1	6.2	0.06	0.08	0.025	0.036	0.247	0.376
KN05	38.0	61.0	0.36	0.26	0.263	0.229	2.634	2.281
	3.6	7.9	0.04	0.04	0.013	0.014	0.129	0.139
KN06	34.0	12.0	0.37	0.63	0.189	0.318	1.891	3.188
	5.8	3.1	0.07	0.10	0.026	0.049	0.260	0.494
KN07	32.6	39.0	0.69	0.65	0.166	0.147	1.668	1.479
	2.9	4.7	0.06	0.07	0.024	0.023	0.243	0.235
KN08	75.0	182.0	0.86	0.72	0.039	0.020	0.399	0.197
	25.5	54.6	0.22	0.37	0.067	0.040	0.686	0.392
KN09	70.0	182.0	0.78	0.57	0.372	0.194	3.722	1.942
	21.0	54.6	0.02	0.05	0.036	0.017	0.364	0.171
KN10[a]	236.2	402.2	0.90	0.85	0.126	0.087	1.267	0.872
	36.1	77.2	0.03	0.05	0.040	0.027	0.401	0.279
KN14	80.0	115.0	0.63	0.39	0.203	0.124	2.029	1.214
	24.6	23.5	0.03	0.05	0.011	0.008	0.115	0.082
KN14B	66.0	155.0	0.79	0.37	0.366	0.124	3.666	1.235
	23.3	55.5	0.02	0.02	0.025	0.007	0.249	0.069
KN15	42.0	46.0	0.55	0.44	0.255	0.205	2.121	1.704
	5.6	17.3	0.04	0.04	0.017	0.017	0.142	0.138
KN15B	54.0	87.4	0.63	0.43	0.332	0.214	2.765	1.784
	4.5	8.8	0.03	0.02	0.017	0.008	0.147	0.138
KN16	83.0	85.0	0.53	0.44	0.218	0.183	2.646	2.231
	12.1	29.2	0.06	0.06	0.018	0.018	0.224	0.225
KN16B	64.0	35.7[b]	0.80	0.61	0.278	0.139	3.388	1.691
	19.2	34.1	0.04	0.07	0.050	0.032	0.623	0.395
Mean	49.3	67.8	0.62	0.54	0.226	0.188	2.263	1.877
SD	17.1	40.2	0.18	0.19	0.142	0.124	1.425	1.238

Value ± SD. The population means were generated by the model using the population mean parameter values shown in Table 4.

[a] KN10 was not included in the calculation of average concentration as this subject was considered to be ketotic.

[b] The calculated concentration of β-OH butyrate was 103.3 $\mu mol\ L^{-1}$.

partment representing liver in their model often exceeds hepatic blood flow.

Our model has one more compartment than that of Cobelli et al. (8). This was necessary to fit data points late in time on the 12 curves from subjects (n = 3) receiving bolus injection of tracer, since we sampled blood for 180 min while Cobelli et al. (8) sampled for only 100 min. A fit by their model to one of our studies is shown in **Fig. 4.** A good fit was obtained to the early data but not to the later data for the slow return of labeled carbon into ketone bodies. Since both models are tracing the carbon atoms that exchange with ketone bodies, our model represents an extension of theirs. In addition, the input of new ketone bodies through the compartment representing the liver gave values inconsistent with our measured blood ketone body concentrations. They averaged the blood concentrations of each ketone body between experiments and used the mean values in their model. We did not assume that the blood concentrations had remained constant, but modeled the tracee concentrations and tracer data from each experiment.

While our model shares some assumptions with other

TABLE 7. A comparison of values calculated using the model (Fig. 3d) and values reported for ketone body kinetics in normal subjects

		Study					
Parameter	Model	1	2	3	4	5	6
N	11	1	9	3	7	24	6
Body weight (kg)							
	71.5	73.3	76	60			
Length of fast (hours)							
	12	20	12	12	12	12	16
Blood concentrations (μmol L^{-1})							
A	49.3	300	129	251	93		
B	67.8	490	132	385	146		
Ac			19	23			
A + B[a]	117.1	790	261	636	239		
Volume of distribution (percent body weight)							
A	14.3		18		10.5		
B	14.3			18	6.7		
(A + B)[b]	14.3		22	16			
Interconversion							
LN_{BA}	0.62						
LN_{AB}	0.54						
Synthesis (μmol min^{-1})							
U_A	64				154		
U_B	88				260		
$U_A + U_B$	152				414		
U_2	206				414		
Hepatic release (μmol min^{-1})							
A							37
B							183
A + B						207	220
PR (μmol min^{-1})							
A	111		349		211		
B	127			312	210		
(A + B)	152	844	524	408			
FCR (min^{-1})							
A	0.226		0.198		0.318		
B	0.188			0.074	0.313		
(A + B)	0.130	0.106	0.120	0.066			
MCR (L min^{-1})							
A	2.263		1.976		2.273		
B	1.877			0.966	1.434		
(A + B)	1.298	1.06	1.672	0.756			

Study	Tracer		Reference
1. Balasse	3-14C A	Primed continuous infusion (2 hr)	(5)
2. Keller et al.	3-14C A	Bolus (14 min) infusion (40 min)	(9)
3. Keller et al.	D-3-14C B	Bolus (14 min) infusion (40 min)	(9)
4. Cobelli et al.	3-14C A, 3-14C B	Bolus, sampling for 100 min	(8)
5. Owen et al.		Arterio-venous difference × blood flow	(49)
6. Vogelberg et al.		Arterio-venous difference × blood flow	(50)

When not given, a body surface area of 1.83 m^2 and a body weight of 70 kg were used to convert literature values to common units.

[a] A + B is the sum of the values calculated individually.

[b] (A + B) refers to the value calculated from total ketone body kinetics.

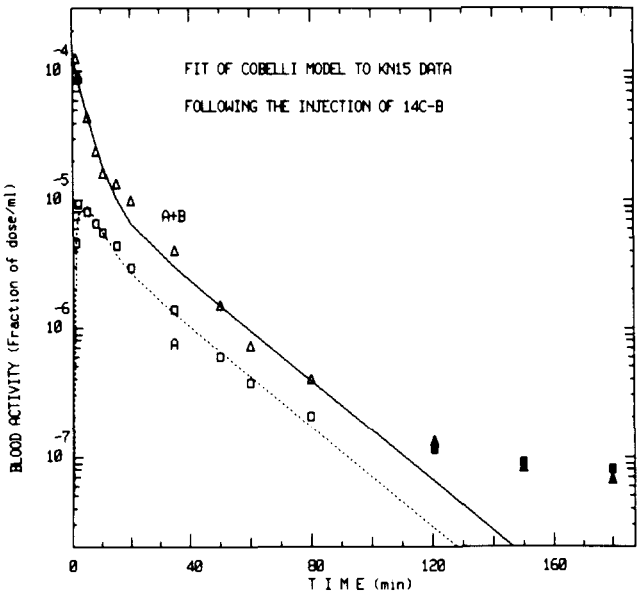

Fig. 4. A fit of the model of Cobelli et al. (8) to one study (KN15A). A good fit was obtained to data up to 100 min after the injection of tracer. The model did not fit the later data and these points were unweighted (shown by the filled-in symbols).

models, such as the rapid equilibration of A and B in extravascular tissues, two other assumptions are introduced. One is that the fractional transfer of A and B into tissues is the same, and this is based on the similar chemistry of the molecules. Although there is variability in this parameter ($L_{2,1}$) between studies, the values are more consistent in the injection studies (Table 4) where early data were available.

The second assumption is that material recycled from the extravascular compartments, re-enters blood in the same ratio as the blood concentrations of A and B. This is based on the premise that the relative blood concentrations are determined by the ratio of NAD/NADH in the liver and tissues. All of the tracer data and most of the tracee data were fitted with this assumption. However, in some studies additional direct inputs of tracee as A or B into blood were necessary (U_1 and U_4, respectively, Table 5). This anomaly could arise if blood tracee concentrations changed during the experiments or, alternatively, if newly synthesized tracee was released into blood in a ratio of A:B that was different from ketone bodies recycling from blood. Differences in the NAD/NADH ratio between various tissues (43) could account for this since the ratio of A to B in tissues is believed to be determined by the NAD/NADH ratio (44)

$$\text{acetoacetate} + \text{NADH} + \text{H}^+ \leftrightarrow \beta\text{-OH butyrate} + \text{NAD}^+. \quad \textit{Eq. 5)}$$

To resolve this problem it would be necessary to label a common precursor and compare the kinetics of ketone bodies synthesized de novo with those of ketone bodies labeled in the blood, i.e., have equivalence of tracer and tracee supply (36).

Distribution volume

The initial space of distribution was 10 L, or 14% of body weight, and represents carbon in ketone bodies in plasma and some extracellular fluid. This value was comparable to others (Table 7). The total (or final) plasma equivalent distribution volume includes compounds that exchange carbon with ketone bodies. Cobelli et al. (8) calculated a total ketone body volume of 52% of body weight after sampling for 100 min. Our value was larger (115% of bodyweight) as our sampling lasted for at least 180 min, and so more of the labeled carbon incorporated into compounds was reincorporated into ketone bodies.

Production and synthesis rates of A and B

The synthesis rate ($U_A + U_B$) is comparable to the hepatic release of A and B determined by others (Table 7). This is consistent with evidence that most of the newly synthesized ketone bodies are released from the liver.

U_2 is the rate of synthesis of new ketone bodies. Some of this was released into blood as A (U_A, Table 5) and some as B (U_B, Table 5). Thus the sum ($U_A + U_B$) is a measure of the rate of release of new ketone bodies into blood as either A or B. It is important to note that this quantity is different from the conventionally calculated quantities PR_A and PR_B (see Table 7), since the latter reflect first entries of particles into blood as A (or B) ignoring the fact that some of these may already have been in blood previously as B (or A).

Although it has recently been contested (8, 39), it has been common practice to calculate the rate of input into the blood of total ketone bodies (defined in our model as $U_A + U_B$) from the ratio of the rate of infusion of the tracer to the combined specific activity plateau (total ketone body radioactivity/total ketone body mass) as originally proposed by McGarry et al. (21). As pointed out by Barton (39), generally the quantity $U_A + U_B$ is not derivable in this manner except when the specific activities of A and B in the blood are equal. However, in tracer studies there is lack of equivalence of tracee and tracer supply (36) since tracer enters blood as label on one ketone body, whereas tracee enters blood as both ketone bodies (called U_A and U_B) and thus the specific activities of A and B in blood will not become equal (26).

An alternative way of studying the relation is in terms of residence times, as these equal the area under the radioactivity curves (28). A condition for the relation to be valid is when the sum of the areas for the injection of A* is the same as it is for B*;

$$LI_{1,1} + LI_{4,1} = LI_{1,4} + LI_{4,4}. \quad \textit{Eq. 6)}$$

This relation states that particles must spend the same length of time in plasma whether they enter as A ($LI_{i,1}$) or B ($LI_{i,4}$) and, as discussed by Cobelli et al. (8), the relation holds when the direct losses from plasma are the same and when the turnover rates in plasma are equivalent. As these conditions were chosen as assumptions in our model ($L_{0,1} = L_{0,4} = 0$ and $L_{2,1} = L_{2,4}$), the value of $U_A + U_B$ is the same as that calculated from the area under the specific activity curves. Thus, as claimed by Balasse and Delcroix (45), the calculation is valid under some conditions; however, it should be stressed that it may not apply in all circumstances.

Utilization rate of A and B

The model can provide insight into the particular mechanism responsible for changes in FCR's (see equation 4). Thus, the change in FCR's observed in repeat studies on the same individuals (KN14, KN15, and KN16, Table 6) were probably due to different mechanisms, as different rate constants altered. For example, the increase in FCR_A in subject KN14 in study 2 is a composite of a decrease in $L_{0,2}$ and in $L_{1,2}$ and an increase in $L_{2,1}$ (Table 4).

The values calculated for FCR, by our group, were higher than some other studies (Table 7), because experiments designed to measure ketone body kinetics in man frequently involve the infusion (46) or injection (47) of the sodium salts of A or B which raises ketone body concentrations. As shown by subjects KN08 and KN10 in Table 6, and observed by Hall et al.,[2] even mild ketosis is associated with a decrease in FCR. By contrast, our values were lower than those of Cobelli et al. (8) but this is probably due to our longer sampling period.

In conclusion, we studied the kinetics of A and B in normal human subjects and developed a compartmental model to account for the observations. The model differs from that proposed by Cobelli et al. (8) in that ours contains an additional compartment to account for the slow interconversion of ketone body carbons with other compounds. In addition, we concluded that in experiments where A and B are labeled directly in blood, i.e., by bolus injection of labeled A or B, and not at the site of synthesis, then separate compartments for the liver and extrahepatic tissues cannot be resolved from measurements of the fraction of dose in the blood as A or as B.

Using the model, the PR and the FCR of A and B can be determined individually. These values can be compared to other physiological states to determine whether there are differences in metabolism. In addition, possible sites of abnormality can be identified by comparing parameter values of normals with ketotic states.

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APPENDIX 1. The fit of one set of data (KN15A) to the models shown in Fig. 3

	Model		
Parameter	3b	3c	3d
min^{-1}			
$L_{0,2}$	0.204 (70)	0.258 (83)	0.253 (31)
$L_{1,2}$	0.055 (151)	0.087 (52)	0.144 (44)
$L_{1,6}$	0.034 (34)		
$L_{1,9}$		$=L_{2,1}$	
$L_{2,1}$	0.301 (14)	0.289 (14)	0.422 (7)
$L_{2,3}$	0.0058 (44)		
$L_{2,4}$	$=L_{2,1}$	$=L_{2,1}$	$=L_{2,1}$
$L_{2,6}$		0.102 (134)	0.140 (33)
$L_{3,2}$	0.133 (55)		
$L_{3,6}$		0.032 (37)	0.028 (17)
$L_{4,2}$	0.113 (62)	0.148 (44)	0.249 (33)
$L_{4,6}$	0.066 (26)		
$L_{4,9}$		$=L_{4,2}$	
$L_{6,1}$	0.094 (23)		
$L_{6,2}$		1.07 (126)	0.525 (39)
$L_{6,3}$		0.0047 (77)	0.0058 (40)
$L_{6,4}$	$=L_{6,1}$		
$L_{9,1}$		0.122 (28)	
$L_{9,4}$		$=L_{9,1}$	

The value (and percent FSD) of each parameter in the model is given.

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