Ketone body kinetics in humans: the effects of insulin-dependent diabetes, obesity, and starvation

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Abstract The kinetics of acetoacetate (A) and β-hydroxybutyrate (B) have been studied following the injection as a pulse or continued infusion of [3-^{14}C]acetoacetate (A*) or [^{14}C]β-hydroxybutyrate (B*) into six newly diagnosed, untreated, ketotic diabetic patients, ten obese subjects in the postabsorptive state, and the ten obese subjects after 1–2 weeks starvation (50 cal per day). Employing a compartmental model of acetoacetate and β-hydroxybutyrate kinetics developed using CONSAM for normal subjects, the rate coefficients ($L_{i,j}$), rates of release of newly synthesized acetoacetate and β-hydroxybutyrate into the blood (U_A, U_B), and fractional removal of each compound (FCR_A and FCR_B) were calculated. Ketone body release into blood ($U_A + U_B$) in diabetic subjects was threefold higher than normal (mean ± SD, 208 ± 118 versus 81 ± 66 μmol min^{-1} m^{-2}) and in obese subjects the rate increased on starvation from 171 ± 70 to 569 ± 286 μmol min^{-1} m^{-2}. In each case most of the increase was in β-hydroxybutyrate. The major change in diabetes and on starvation of the obese subjects was in the rate coefficient for removal of ketone bodies. Normally 0.168 ± 0.109 min^{-1}, it was 0.055 ± 0.040 min^{-1} in the diabetic patients and fell from 0.066 ± 0.040 to 0.027 ± 0.019 min^{-1} in the obese subjects on starvation. In normal subjects, FCR_A was similar to FCR_B (0.226 ± 0.142 versus 0.188 ± 0.124 min^{-1}). However, in diabetics, FCR_A was 0.074 ± 0.044 and FCR_B was 0.050 ± 0.034 min^{-1} and both were lower than normal. On starvation of obese subjects, FCR_A fell from 0.199 ± 0.047 to 0.089 ± 0.035 min^{-1}, whereas FCR_B fell from 0.141 ± 0.040 to 0.033 ± 0.012 min^{-1}. Therefore, the removal of β-hydroxybutyrate was impaired more than that of acetoacetate in all patients. Our results confirm previous observations that ketosis is associated with high rates of ketogenesis and a decrease in fractional clearance. In addition, we found that in diabetes, obesity, and in obese subjects following starvation, most of the increased synthesis was in β-hydroxybutyrate and that the clearance of β-hydroxybutyrate decreased more than that of acetoacetate.—**Hall, S. E. H., M. E. Wastney, T. M. Bolton, J. T. Braaten, and M. Berman.** Ketone body kinetics in humans: the effects of insulin-dependent diabetes, obesity, and starvation. *J. Lipid Res.* 1984. **25:** 1184–1194.

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The concentrations of ketone bodies acetoacetate (A) and β-OH butyrate (B) are normally in the order of 0.2 mM but can rise 50-fold during starvation, and even further in pathological states when glucose metabolism is impaired such as diabetes mellitus and glycogen storage disease (see 1). Under these conditions ketone bodies are of vital importance, providing energy for continued brain function (2).

Despite this, quantitative information concerning the kinetics of acetoacetate and β-OH butyrate metabolism in man in vivo has been difficult to obtain. Tracer studies, in particular, have been beset by difficulties in interpretation (for discussion, see ref. 3), and have tended to rely on calculation of combined delivery rates of ketone bodies to the blood and their combined metabolic clearance rates. However, more detailed quantitative information is required in order to understand the regulation of the metabolism of these ketone bodies, especially under conditions where they may be metabolized at different rates.

The purpose of the experiments reported here was to make a quantitative investigation of acetoacetate and β-OH butyrate metabolism in man under the metabolic stresses of insulin-requiring diabetes and starvation of obese subjects. Data obtained following the continuous infusion or pulse injection of radiolabeled acetoacetate or β-OH butyrate have been analyzed using a mathematical model developed earlier for their metabolism in normal humans (3).

METHODS

Subjects

The potential risks were explained to all participants and their informed, written consent was obtained. Nine

Abbreviations: In some instances, acetoacetate and β-OH butyrate are designated as A and B, respectively. Terminology associated with the kinetic model is in Table 2.

[1] Dr. Berman died August 12, 1982.

healthy subjects with no family history of diabetes and of normal wt ht^{-2} ratio (18–25 kg m^{-2}, (4)) acted as controls, and are reported in (3).

Ten obese patients, with a mean wt ht^{-2} ratio of 50 ± 3.6 kg m^{-2}, who had been admitted to the metabolic unit of the Ottawa Civic Hospital for therapeutic starvation, volunteered to take part in the experiments. They were studied in the postabsorptive state, and again after 1–2 weeks (mean 12 ± 2 days) of near starvation, before food was reintroduced. During the starvation period the subjects received 50 cal and one multiple vitamin capsule per day and water intake was not controlled. None of the obese subjects had any history of diabetes or elevated fasting blood sugar and they were generally healthy, with the exception of patients O07 and O08 who had epilepsy and were treated with dilantin (100 mg tid).

Six otherwise healthy diabetic patients, diagnosed at the Diabetic Clinic of the Ottawa Civic Hospital, were also studied. They were judged to be, on clinical grounds, insulin-requiring although some may have been Type II diabetics. They were studied on presentation before any treatment was begun.

Pertinent clinical details are given in **Table 1** together with the mean values for normal subjects, reported in detail earlier (3).

Tracers

The tracers used were D[3-^{14}C]acetoacetate (A*) and [3-^{14}C]β-hydroxybutyric acid (B*) (Amersham Corp.). They were prepared as described previously (3).

Experimental protocol

All subjects were studied in the postabsorptive state. They were instructed not to eat after 9 PM of the day prior to the study although no stipulations were made concerning the meals taken prior to this time.

The subjects were warm and semi-supine in bed, and most slept throughout the experiment. A polyethylene cannula was introduced into an antecubital vein in each arm. The tracer was infused (or injected) through one

TABLE 1. Details of subjects

			Metabolic State				
			Postabsorption		Starvation		
Subject	Sex	Age	Weight	BSA[a]	Length	Weight	BSA
		yr	*kg*	m^2	*days*	*kg*	m^2
Normal (n = 11)							
Mean		41	71.5	1.86			
SD		14	10.7	0.19			
Diabetic (n = 6)							
D03	M	37	79.1	1.80			
D04	M	36	74.1	1.87			
D05	M	29	76.3	1.93			
D06	M	31	60.3	1.72			
D07	F	49	48.4	1.51			
D08	M	37	55.0	1.67			
Mean		36	65.5	1.75			
SD		7	12.7	0.15			
Obese (n = 10)							
O02	F	25	82.6	1.90	14	76.8	1.84
O03	F	38	99.2	1.99	13	94.5	1.95
O06	M	19	187.4	2.89	13	178.1	2.84
O07	F	39	86.2	1.86	15	81.4	1.83
O08	M	34	148.2	2.53	10	142.2	2.49
O09	M	27	178.9	2.72	13	167.6	2.72
O10	F	40	135.5	2.33	8	132.0	2.31
O11	M	38	171.9	2.83	14	161.6	2.76
O12	M	29	100.8	2.21	13	94.5	2.15
O13	F	43	119.3	2.30	13	112.9	2.25
Mean		33	131.0	2.36	12	124.2	2.31
SD		8	39.2	0.38	2	37.3	0.38

[a] Body surface area, calculated from the formula of Du Bois (see ref. 36).

TABLE 2. Nomenclature

Expression	Name	Definition
FCR_A[a]	Fractional catabolic rate	The fraction of blood acetoacetate that is cleared per unit of time
$L_{i,j}$	Rate constant	Fractional flow into compartment i from compartment j, per unit time
$L_{0,j}$	Fractional loss	Fraction of material lost from compartment j per unit time
LN_{AB}	Fractional transfer	The fraction of blood β-OH butyrate that is converted to blood acetoacetate.
MCR_A	Metabolic clearance	The volume of blood cleared of acetoacetate per unit of time
PR_A	Production rate	The rate of first appearance of acetoacetate in the blood
$R_{i,j}$	Flow rate	Tracee flow into compartment i from compartment j, per unit time
$R_{0,j}$	Utilization	Rate that tracee is lost from the system, per unit time
U_i	Synthesis	Entry of tracee into compartment i, per unit time
U_A	Synthesis of A	Rate of appearance of acetoacetate in the blood for the first time, as acetoacetate

[a] A and B can be interchanged in these expressions.

catheter and blood samples were taken from the contralateral vein.

Two of the obese subjects (O12 and O13) received [3-^{14}C]acetoacetate as a single injection before and after starvation while two others (O02 and O03) received [^{14}C]β-OH butyrate by pulse injection. All the other subjects received [3-^{14}C]acetoacetate as a continuous infusion.

Following the injection of 100 μCi of the labeled material, blood samples were taken at 1, 2, 4, 7, 9, 15, 20, 35, 50, 60, 80, 120, 150, and 180 min. When the tracer was infused (at approximately 0.29 μCi 0.0765 ml^{-1} min^{-1}), blood samples were taken at 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, and 240 min.

Chemical methods

The blood concentrations of acetoacetate and β-OH butyrate were measured enzymically (5) within one day of the experiments; their specific activities were determined in each sample by the method of Bates, Krebs, and Williamson (6) as described previously (3).

Samples from experiments where the tracer was injected were lyophilized to remove all labeled acetone. In seven of the infusion experiments, some acetone was removed by bubbling nitrogen through the samples for 30 min (7). However, adding [^{14}C]acetone (Ac*) to blood filtrates showed that acetone was only partially (53.0 ± 5%) removed by this procedure (8).

Insulin concentration

Serum insulin was measured in subjects who received the tracer by infusion (9) and all samples were measured in the same assay using human insulin standards.

Model description and kinetic analysis

The data were analyzed using a model, developed previously for normal subjects (3), using the SAAM (10) and CONSAM (11, 12) programs on a VAX 11/780 computer (Digital Equipment Corp., MA). Terminology associated with the model is given in **Table 2.**

The model (**Fig. 1**) consists of blood acetoacetate (compartment 1) and blood β-OH butyrate (compartment

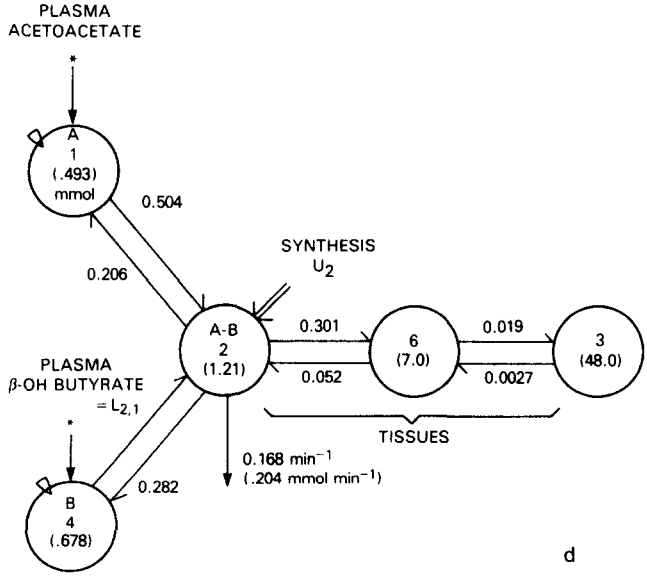

Fig. 1. The model proposed for acetoacetate (A) and β-OH butyrate (B) kinetics (3). A star (*) denotes site of tracer input, while the double arrow denotes input of tracee. The rate constants $L_{i,j}$, (min^{-1}), masses (mmol), and utilization rate (mmol min^{-1}) for normal subjects (3) are shown.

4). Blood acetoacetate and β-OH butyrate are interconverted in a rapidly turning over (2 min) compartment (compartment 2) that includes the liver and some extrahepatic tissues. Ketone body carbons exchange with other, more slowly turning over, compounds and these are represented by compartments 3 and 6.

Several assumptions were introduced in developing the model. The first assumption was that acetoacetate and β-OH butyrate have the same probability of moving from blood into extravascular spaces ($L_{2,1} = L_{2,4}$), since both ketone bodies are small molecules and probably move by diffusion. The second assumption was that acetoacetate (A) and β-OH butyrate (B) are released from the extravascular compartments into blood in the same ratio as exists in the blood. Thus

$$L_{1,2}/(L_{1,2} + L_{4,2}) = \text{Blood A}/(\text{Blood A} + \text{B}). \quad \textit{Eq. 1)}$$

This is based on the premise that the relative concentrations of acetoacetate and β-OH butyrate in blood reflect those in the tissues.

One feature of our model that differs from the model developed by Cobelli et al. (13), to fit data from similar experiments, is that we do not represent the liver as a single pool. It was not possible, from the blood tracer data collected in our experiments, to define a pool that rapidly interconverted acetoacetate and β-OH butyrate and whose kinetics were consistent with known hepatic physiology, i.e., the flow rates into a liver compartment would exceed the circulatory flow to the liver (3). It was concluded that some rapid interconversion occurred in extrahepatic tissues and a minimal model was chosen to describe the kinetics of acetoacetate and β-OH butyrate in which all rapid interconversion occurred in one extravascular compartment, compartment 2.

Entry of new material into the system was into the rapid compartment. However, one inconsistency of our model was that the blood tracee concentrations were not always consistent with the tracer data. Thus it was necessary, in some experiments, to have additional input of tracee as acetoacetate (U_1) or as β-OH butyrate (U_4). This is probably a result of introducing the tracer, as [3-^{14}C]acetoacetate or [^{14}C]β-OH butyrate, into blood. Ketone bodies are synthesized extravascularly and label introduced into blood may not precisely follow the kinetics of the native material. Introducing the label on a precursor may resolve this inconsistency.

In fitting the data from the infusion experiments, the rate constants for the slower compartments (compartments 3 and 6) were weighted by the values for normals (Fig. 1). In addition, as discussed under Methods, some [^{14}C]acetone (Ac*) was measured along with labeled acetoacetate in samples from the infusion experiments. The kinetics of acetone determined from published experiments (14) were included in the model as described in our earlier paper (3) and, in experiments where acetone was not completely removed, some of the acetone pool was included with labeled blood acetoacetate in fitting the data.

The rate constants of the model ($L_{i,j}$) were adjusted to obtain a fit of the observed tracer and the steady state tracee data for each subject and the tracee inputs (U_i), flow rates ($R_{i,j}$), fractional catabolic rates (FCR), and metabolic clearance rates (MCR) were calculated.

The rate of appearance of each ketone body in the blood for the first time as acetoacetate (U_A) or as β-OH butyrate (U_B) was calculated by the following equations:

$$U_A = U_1 + U_2 \bullet L_{1,2}/(L_{4,2} + L_{1,2} + L_{0,2}) \quad \textit{Eq. 2a)}$$

$$U_B = U_4 + U_2 * L_{4,2}/(L_{4,2} + L_{1,2} + L_{0,2}). \quad \textit{Eq. 2b)}$$

As discussed elsewhere (3) these rates differ from the production rates of acetoacetate or β-OH butyrate, as production rates measure acetoacetate or β-OH butyrate released into the blood for the first time in that form plus a fraction of the new material released as the other ketone body.

The fraction of blood acetoacetate converted to blood β-OH butyrate (LN_{BA}) and the fraction of β-OH butyrate converted to acetoacetate (LN_{AB}) were determined for this model (3) as:

$$LN_{BA} = L_{4,2}/(L_{4,2} + L_{0,2}) \quad \textit{Eq. 3a)}$$

$$LN_{AB} = L_{1,2}/(L_{1,2} + L_{0,2}). \quad \textit{Eq. 3b)}$$

These fractions can also be derived from ratios of areas under specific activity curves (see 3).

The final parameter values were determined by a least squares iterative procedure (10) and the goodness of fit was assessed by minimizing the sum of squares, the lack of consistent deviations of the calculated fit about the data, and by well-determined parameters.

Statistical comparisons were made between groups using the Student's *t*-test, while a paired *t*-test was used for the studies in obese subjects in the postabsorptive and starved states.

RESULTS

The mean values of the normal studies are presented here for comparison with the studies in the diabetic and obese groups. Detailed results of the normal subjects are presented in an earlier paper (3).

The observed data for infusion experiments from each group (**Fig. 2**) and for injection experiments on two obese subjects (**Fig. 3**) are shown, together with the model generated curves.

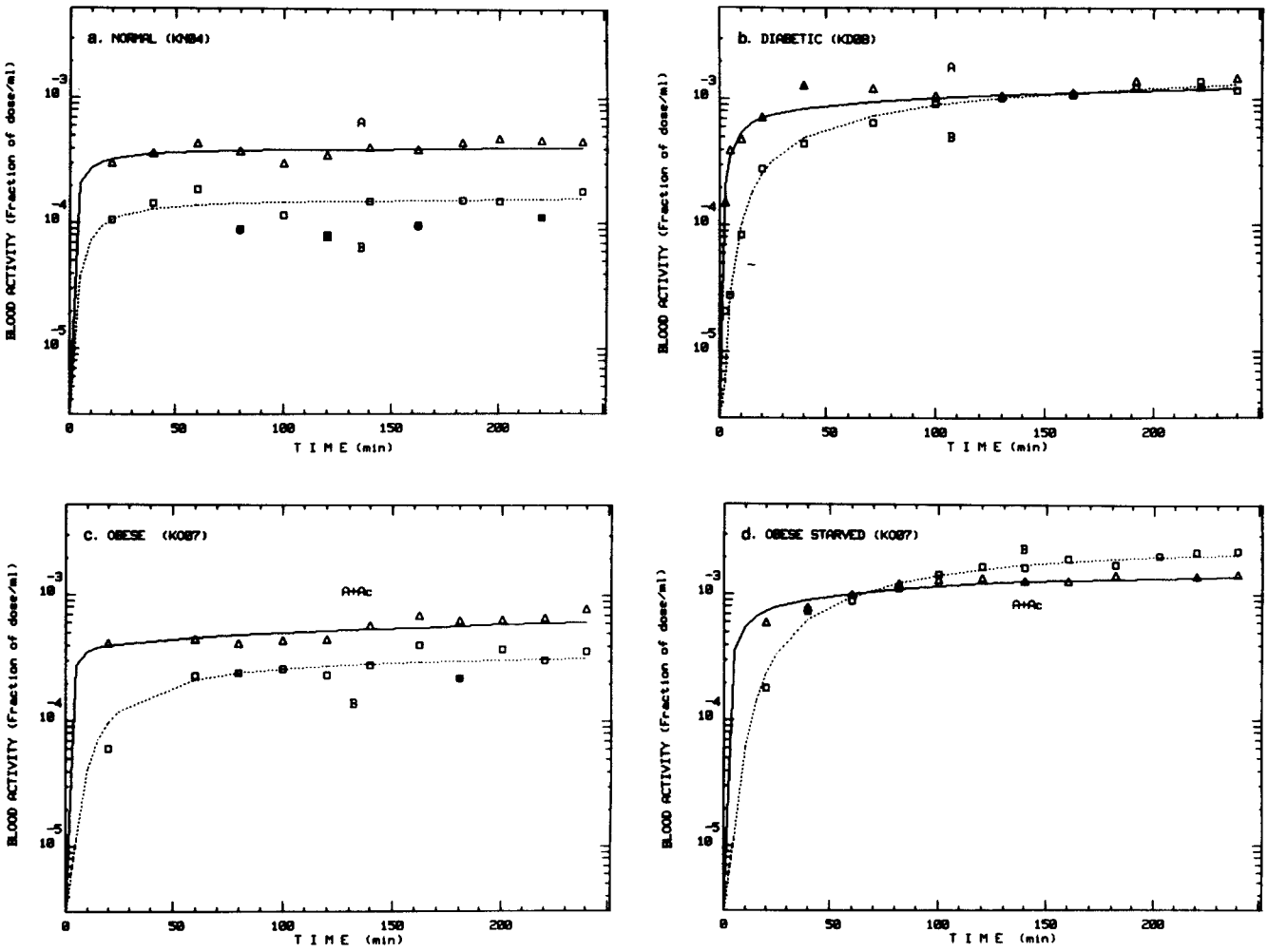

Fig. 2. The observed data and model generated fits to the blood activity of acetoacetate (A) and β-OH butyrate (B) during an infusion of [^{14}C]acetoacetate into a) a normal subject, b) a diabetic, c) an obese subject in the postabsorptive state, and d) the same obese subject after a period of starvation. The solid points were given zero weight in the data fitting. In some studies, acetone (Ac) was measured along with A.

Blood concentrations

The mean blood concentrations of acetoacetate and β-OH butyrate are shown in **Table 3.** The diabetic patients were a heterogeneous group, as can be seen from their plasma insulin concentrations (Table 3), and their ketone body concentrations ranged from 4- to 75-fold higher than normal. The total ketone body concentration of obese subjects was 3-fold higher than normals (0.42 vs 0.12 mM) and starvation of the obese subjects increased the concentrations 12-fold (0.42 to 5.01 mM). Older subjects tended to have higher concentrations and there was a significant correlation between age and ketone body concentration.

The ratio of acetoacetate to acetoacetate + β-OH butyrate varied between groups. It was reduced from 0.42 in normals to 0.27 in diabetics, 0.36 in obese subjects, and 0.21 in obese subjects following starvation. Higher ketone body concentrations were therefore associated with an increased proportion of β-OH butyrate.

Rate constants

The differences in the kinetics between the normal, diabetic, and obese subjects could be explained by changes in four rate constants, $L_{2,1}$, $L_{1,2}$, $L_{4,2}$, and $L_{0,2}$ (**Table 4**). The rate of movement of acetoacetate from the blood into tissues ($L_{2,1}$) was significantly lower in the diabetic patients than normal, while the ratio of $L_{1,2}/L_{4,2}$ reflected the change in acetoacetate:β-OH butyrate blood concentrations (Eq. 1). The fraction lost by oxidation and excretion ($L_{0,2}$) was significantly reduced in diabetes, obesity, and obese subjects on starvation.

The decreased loss from the system was coupled with an increase in the interconversion between the two

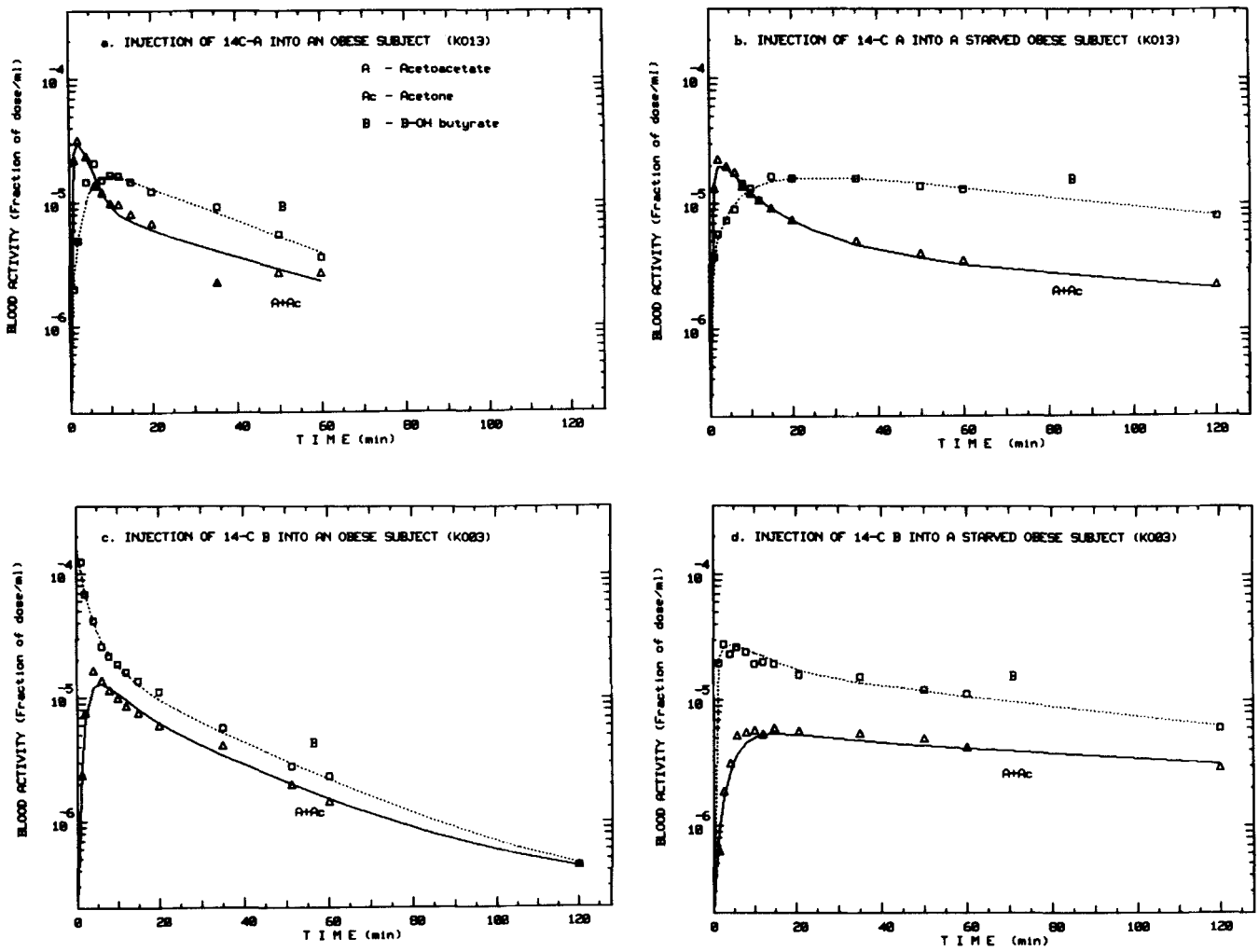

Fig. 3. The observed data and model generated fits to blood activity of acetoacetate (A) and β-OH butyrate (B) in one obese subject following the injection of [^{14}C]acetoacetate in a) the postabsorptive state and b) after a period of starvation, and a second obese subject after an injection of [^{14}C]β-OH butyrate in c) the postabsorptive state or d) after a period of starvation. In experiments a, b, and d, the tracer did not directly enter the blood vessel. The filled-in symbols were given zero weight during the data fitting.

ketone bodies (Table 4). In all groups, LN_{BA} was higher than LN_{AB}, showing that a larger fraction of acetoacetate was converted to β-OH butyrate than vice versa.

Synthesis rates

Most of our obese subjects were extremely overweight (Table 1). There is a problem in comparing data from individuals of different weights due to their differing body composition and we chose to normalize all metabolic data to body surface area since body composition measurements were not available.

The synthesis rate of ketone bodies (U_2) and the rate of release into blood ($U_A + U_B$) in the diabetic patients and obese subjects was about double the rate of normals (**Table 5**). In four of the obese studies (O13 before, and O10, O11, and O13 after starvation), new ketone bodies appeared in blood only in the form of β-OH butyrate (U_4). In the other obese subjects, U_2 and the rate of appearance of ketone bodies in the blood were significantly increased by starvation.

It can be seen (Table 5 and **Fig. 4**) that the higher ketone body production rate in diabetes was mainly in the form of β-OH butyrate. Before starvation neither U_A nor U_B were significantly raised above the normal rate (Table 5). However, following starvation of the obese subjects, U_B increased significantly while U_A remained essentially unchanged.

Rates of utilization

Despite the fall in fractional loss ($L_{0,2}$) of ketone bodies in all groups (Table 4), the rate of ketone body removal was significantly higher than normal in the

TABLE 3. The blood concentrations of acetoacetate (A), β-OH butyrate (B), glucose, and insulin

Subject	Metabolic State: Postabsorption A	B	Glucose	Insulin	Starvation A	B	Glucose	Insulin
	mM	*mM*	*mM*	*μU ml*$^{-1}$	*mM*	*mM*	*mM*	*μU ml*$^{-1}$
Normal (n = 11)								
Mean	0.05	0.07	4.8	7.3				
SD	0.02	0.04	0.6	1.4				
Diabetic (n = 6)								
D03	0.21	0.26	16.2	12.4				
D04	0.14	0.29	14.5	9.2				
D05	0.23	0.38	18.8	3.3				
D06	0.43	0.76	13.1	2.8				
D07	2.08	6.99	16.0	4.5				
D08	0.94	2.08	14.1	4.7				
Mean	0.67[a]	1.79	15.5[b]	6.2				
SD	0.75	2.63	2.0	3.8				
Obese (n = 10)								
O02	0.15	0.28	5.5		0.77	3.70	3.3	
O03	0.22	0.34	5.6		1.11	3.97	3.8	
O06	0.12	0.18	5.1	26.4	1.22	4.63	3.9	14.8
O07	0.09	0.24	3.9	6.0	1.00	3.08	3.5	5.8
O08	0.20	0.47	3.7	22.1	0.99	3.68	3.9	13.9
O09	0.23	0.39	5.1	24.8	1.26	5.14	4.8	24.8
O10	0.12	0.12	4.8	14.2	0.65	1.46	3.6	4.4
O11	0.17	0.29	5.3	34.8	1.47	4.34	3.5	22.5
O12	0.06	0.07	5.6		1.21	5.48	3.7	
O13	0.09	0.30	5.5		0.76	4.23	4.2	
Mean	0.15[b]	0.27[b]	5.0	21.4[b]	1.04[c]	3.97[c]	3.82[c]	14.4[d]
SD	0.06	0.12	0.7	10.0	0.26	1.13	0.43	8.3

[a] Differs from normal ($P < 0.05$) using the *t*-test.
[b] Differs from normal ($P < 0.01$) using the *t*-test.
[c] Differs from obese in postabsorptive state ($P < 0.01$) using the paired *t*-test.
[d] Differs from obese in postabsorptive state ($P < 0.05$) using the paired *t*-test.

TABLE 4. Rate constants and fractional interconversion of ketone bodies

Group	Rate Constant[a] $L_{1,2}$	$L_{4,2}$	$L_{2,1}$	$L_{0,2}$	Fractional Interconversion[b] LN_{BA}	LN_{AB}
	min^{-1}					
Postabsorption						
Normal (n = 11)						
Mean	0.206	0.282	0.504	0.168	0.62	0.54
SD	0.128	0.152	0.222	0.109	0.18	0.19
Diabetic (n = 6)						
Mean	0.159	0.289	0.248	0.055	0.82	0.71
SD	0.108	0.125	0.117	0.040	0.08	0.11
Obese (n = 10)						
Mean	0.147	0.217	0.489	0.066	0.69	0.56
SD	0.235	0.241	0.198	0.040	0.09	0.12
Starvation						
Obese (n = 10)						
Mean	0.108	0.332	0.373	0.027	0.89	0.71
SD	0.100	0.241	0.205	0.019	0.05	0.12

[a] The rate constant $L_{i,j}$ represents the fraction of compartment j that moves to compartment i per unit time.
[b] LN_{BA} is the fraction of blood A which is converted to blood B, while LN_{AB} is the fraction of blood B converted to blood A.

TABLE 5. Synthesis rates of ketone bodies[a]

Group	Metabolic State: Postabsorption U_2	U_A	U_B	$U_A + U_B$	Starvation U_2	U_A	U_B	$U_A + U_B$
	$\mu mol\ min^{-1}\ m^{-2}$							
Normal (n = 11)								
Mean	110	34	47	81				
SD	105	26	43	66				
Diabetic (n = 6)								
D03	153	61	76	137				
D04	191	47	99	145				
D05	184	58	97	155				
D06	170	58	95	153				
D07	475	105	339	444				
D08	231	68	146	214				
Mean	234[b]	66	142[b]	208[b]				
SD	120	20	99	118				
Obese (n = 10)								
O02	35[c]	217	22	239	283	54	22	278
O03	370	112	181	293	1353	297	968	1266
O06	239	67	98	165	682	133	508	641
O07	252	44	121	166	711	142	439	581
O08	234	56	131	187	420	81	302	384
O09	290	77	130	207	740	133	524	658
O10	100	45	46	91	0[d]	0[d]	224	224
O11	254	73	116	190	0[d]	0[d]	514	514
O12	74	25	30	55	647	105	479	584
O13	0[d]	0[d]	112	112	0[d]	0[d]	561	561
Mean	185	71	99	171[b]	483[e]	94	454[f]	569[f]
SD	123	59	51	70	432	91	247	286

[a] U_2 is the synthesis rate of ketone bodies, U_A is the rate of release of newly synthesized acetoacetate into blood, and U_B is the release of β-OH butyrate.
[b] Differs from normal ($P < 0.05$) using the *t*-test.
[c] Additional material appeared in blood as acetoacetate, $U_1 = 210\ \mu mol\ min^{-1}\ m^{-2}$.
[d] All new material appeared in blood as β-OH butyrate, U_4.
[e] Differs from obese in postabsorptive state ($P < 0.05$) using the paired *t*-test.
[f] Differs from obese in postabsorptive state ($P < 0.01$) using the paired *t*-test.

diabetic and obese patients in the postabsorptive state and increased significantly in the obese subjects following starvation (**Table 6**).

Normally, FCR_A was similar to FCR_B (0.226 ± 0.142 vs. $0.188 \pm 0.124\ min^{-1}$, **Fig. 5** and Table 6). However, both were lower than normal in the diabetic patients; moreover FCR_B was lower than FCR_A (Table 6). Before starvation, obese subjects had normal FCR_A, but FCR_B was lower than FCR_A. Both FCR's decreased in the obese subjects on starvation. FCR_A fell by 55% while FCR_B was reduced by 76%.

The metabolic clearance rates (MCR) of acetoacetate and β-OH butyrate (MCR_A, MCR_B) were related to FCR by a factor of 10 since the distribution volume of acetoacetate and of β-OH butyrate was 10 liters. Thus MCR was lower than normal in diabetic patients and in the starved obese group, and in all patient groups MCR_B was lower than MCR_A.

DISCUSSION

In order to examine ketone body metabolism and its regulation, we have studied the kinetics of ketone bodies in several different states: in diabetic patients and in obese subjects in the postabsorptive state and in the same obese subjects after a 2-week period of starvation. We have calculated synthesis and utilization rates of acetoacetate and of β-OH butyrate separately and have examined the differences in ketone body metabolism in diabetes, obesity, and starvation by using a compartmental model (3).

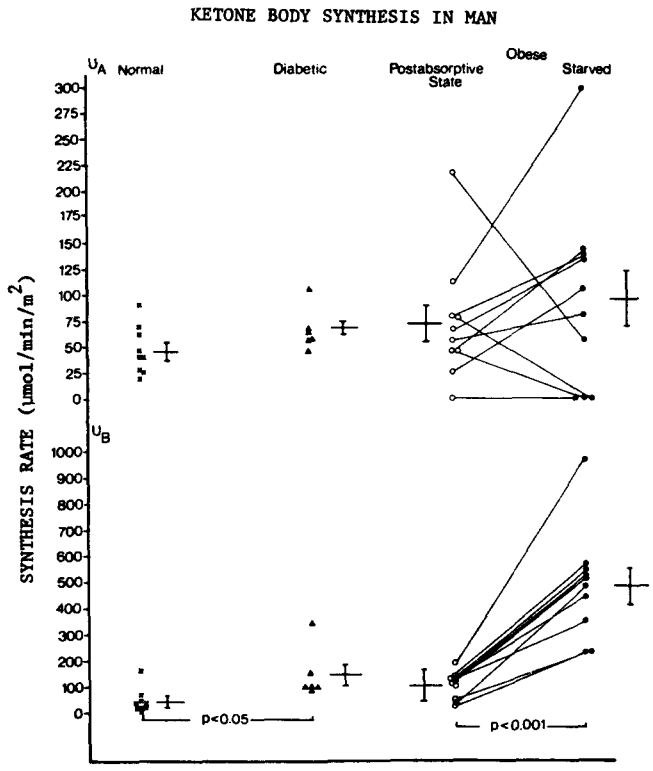

Fig. 4. The rate of appearance of acetoacetate (U_A, upper panel) and the rate of appearance of β-OH butyrate (U_B, lower panel) into blood (μmol min^{-1} m^{-2}) in normal, diabetic, obese, and obese starved subjects. Lines and bars denote mean ± SEM.

Ketone body synthesis rates

It has been suggested that increased ketogenesis is the major factor responsible for the development of ketoacidosis (15, 16). We found the mean synthesis rate (U_A + U_B, Table 5) in the diabetic patients and obese subjects to be double the rate of normals and, while several studies have shown no (15) or only a slight increase (17, 18) in ketogenesis when starvation was prolonged beyond 2 or 3 days, our obese starved patients demonstrated, on average, a threefold rise over the rate in the postabsorptive state.

The increases we measured following a period of starvation (Table 5) were larger than those measured in most other studies (15, 19) and this is probably because we first mesured the subjects in the postabsorptive state, whereas other studies (18) did not begin until the patients had already been starved for 2–3 days. In addition, we studied the same subjects before and after starvation. Our values were higher than those of Owen et al. (15, 19) determined by arterio-venous difference and splanchnic blood flow, as their measurements probably included consumption of ketone bodies by extrahepatic splanchnic tissues. We concluded that in obese subjects, as in diabetics, increased synthesis appears to contribute to the development of ketosis.

In our experiments, where only blood tracer activity was measured, we were unable to differentiate between the sites of ketogenesis. Although the liver is considered to be the main site of synthesis, the muscle is also capable of de novo synthesis and because of its mass, it may be an important contributor under some conditions (20, 21). However, the mean rate of entry of acetoacetate and β-OH butyrate (1314 μmol min^{-1}) in the starved subjects is close to the maximum rate of ketogenesis (1618 μmol min^{-1}) calculated for the liver (22).

The use of the model allowed us to determine the contributions of acetoacetate and β-OH butyrate separately. We found that the increases in ketone body appearance in the blood in diabetes and starvation were almost entirely in the form of β-OH butyrate (Fig. 4). This agrees with the observation that net ketone body release across the splanchnic bed changed in favor of β-OH butyrate when starvation was prolonged from 3

TABLE 6. Rate of utilization and clearance of ketone bodies from blood (mean ± SD)

		Clearance[a]			
	Utilization	FCR_A	FCR_B	MCR_A	MCR_B
	μmol $min^{-1}m^{-2}$	*min^{-1}*		*L min^{-1}*	
Postabsorption					
Normal (n = 11)	110.7 ± 105.9	0.226 ± 0.142	0.188 ± 0.124	2.26 ± 1.42	1.88 ± 1.24
Diabetic (n = 6)	218.8 ± 87.4[b]	0.074 ± 0.044[c]	0.050 ± 0.034[c]	0.74 ± 0.41[c]	0.50 ± 0.34[c]
Obese (n = 10)	210.1 ± 93.2[b]	0.199 ± 0.047	0.141 ± 0.040	1.99 ± 0.47	1.41 ± 0.40
Starvation					
Obese (n = 10)	567.5 ± 256.2[d]	0.089 ± 0.035[d]	0.033 ± 0.012[d]	0.89 ± 0.35[d]	0.33 ± 0.12[d]

[a] FCR is the fraction of blood acetoacetate (FCR_A) or β-OH butyrate (FCR_B) cleared per unit time while MCR is the volume of blood cleared of acetoacetate (MCR_A) or β-OH butyrate (MCR_B) per unit time.
[b] Differs from normal ($P < 0.05$) using the *t*-test.
[c] Differs from normal ($P < 0.01$) using the *t*-test.
[d] Differs from obese in postabsorptive state ($P < 0.01$) using the paired *t*-test.

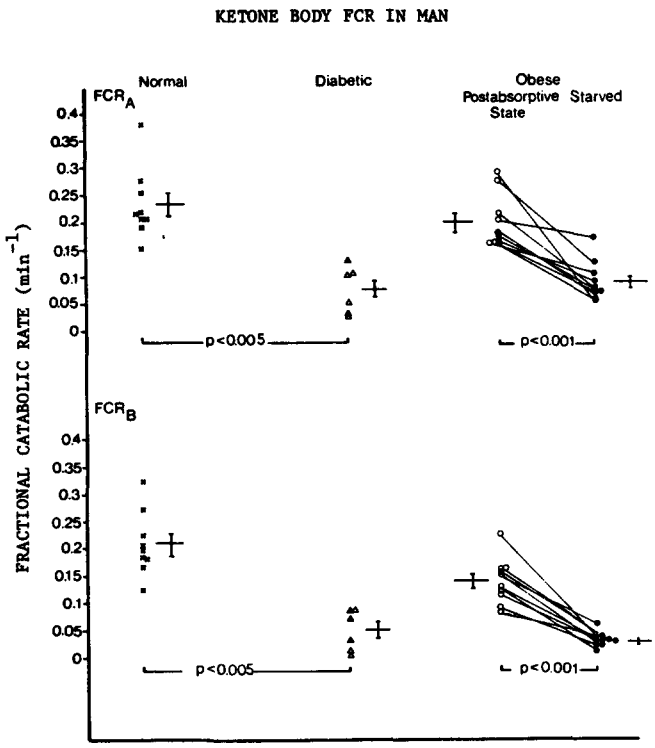

Fig. 5. The fractional catabolic rate of acetoacetate (FCR_A, upper panel) and β-OH butyrate (FCR_B, lower panel) removed from the system (min^{-1}) in normal, diabetic, obese, and obese starved subjects. Lines and bars denote mean ± SEM.

days to 5–6 weeks in obese subjects (23) and in a group of diabetic patients from whom insulin was withdrawn for 24 hr (24). This change from acetoacetate to β-OH butyrate production can also be induced by ethanol infusion (25) and it is, therefore, likely to be linked to a more reduced redox state, presumably in the liver.

Ketone body uptake and utilization

We observed that the rate of entry of ketone bodies into cells ($L_{2,1}$) was low in diabetics compared with normals (Table 4). Ketone bodies enter most tissues as the free acids (26–28) and uptake is not considered to be rate-limiting, although a carrier mechanism has been postulated for the brain (29, 30). Our results suggest that ketone bodies may enter cells more slowly in the diabetic patients and the reason for this difference is not clear.

Ketone bodies are oxidized by most tissues (except liver) to provide energy (31), and are used as substrates for lipogenesis in adipose tissue (32). In conditions such as starvation and diabetes, utilization by both these processes decreases (21, 23, 33, 34), and we measured lower values for the fractional rate of utilization in all patient groups, ($L_{0,2}$ Table 4). Our studies in the patient groups agree with those of Balasse (17) that show that high ketone body levels are associated with a lower clearance. The low value of $L_{0,2}$ in the diabetic group is interesting considering the mild ketosis of the majority.

Depsite the decline in fractional clearance of ketone bodies in all groups, their rate of removal was higher than normal (Table 6). The rate in diabetics and obese subjects was twice the rate of normal subjects and was fivefold higher in obese subjects on starvation (Table 6). The increased rate of utilization coupled with the decrease in clearance supports the suggestion that ketone bodies are utilized by a rate-limiting, or saturable, process (28). As a decrease in clearance was also observed in two normal subjects with elevated blood ketone body concentrations (3) it appears that this process may be a normal regulatory mechanism. The nature of the mechanism may differ between the physiological states.

We found that FCR_B decreased more than the FCR_A in the diabetics and obese groups (Table 6). One explanation of this is that preferential oxidation of free fatty acids by tissues like muscle (35) would shift the NAD:NADH ratio in favor of NADH and this would restrict the entry of β-OH butyrate into oxidative pathways.

In conclusion, our studies have examined the metabolism of acetoacetate and β-OH butyrate in various physiological states. We have shown that ketone body synthesis in diabetic patients and in obese subjects is about double the rate of normals, and that on starvation the rate more than doubles again. Our results agree with earlier studies (17, 18) which showed that ketosis is associated with increased ketone body synthesis. Coupled with this increased production was a substantial decrease in the fractional clearance of ketone bodies. These findings support the suggestion that ketone body utilization is a saturable process (28). This mechanism appears to operate in normal individuals although the degree to which it operates may differ between physiological states. We have shown that both the increased release of new material into blood and decreased clearance from blood were due more to changes in the metabolism of β-OH butyrate than that of acetoacetate.

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