Rapid Intravenous Sodium Acetoacetate Infusion in Man

METABOLIC AND KINETIC RESPONSES

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ABSTRACT The metabolic and kinetic responses to rapidly intravenously administered sodium acetoacetate (1.0 mmol/kg body wt) was studied after an overnight fast in 12 male and female adults weighing between 88 and 215% of average body weight. Blood was obtained before, during, and after the infusion for determination of circulating concentrations of immunoreactive insulin, glucose, acetoacetate, β-hydroxybutyrate and free fatty acids. In three obese subjects the studies were repeated after 3 and 24 days of total starvation.

After the overnight fast acetoacetate rose rapidly reaching a peak concentration at the end of the infusion; β-hydroxybutyrate concentrations also increased rapidly and exceeded those of acetoacetate 10 min postinfusion. Total ketone body concentration at the end of the infusion period was comparable to that found after prolonged starvation. After the initial mixing period, acetoacetate, β-hydroxybutyrate and total ketone bodies rapidly declined in a parallel manner. There were no obvious differences between the subjects with regard to their blood concentrations of ketone bodies. The mean plasma free fatty acid concentration decreased significantly during the 20th to 90th min postinfusion period; for example the control concentration of 0.61 mmol/liter fell to 0.43 mmol/liter at 60 min. In the three obese subjects studied repeatedly, fasting plasma free fatty acids decreased with acetoacetate infusion from 0.92 to 0.46 mmol/liter after the 3 day fast and from 1.49 to 0.71 mmol/liter after the 24 day fast. Acetoacetate infusion caused no changes in blood glucose concentration after an overnight fast. However, in the three obese subjects restudied after 3- and 24-day fasts blood glucose decreased, respectively, from 3.49 to 3.22 mmol/liter and from 4.07 to 3.49 mmol/liter. The mean serum insulin concentration in all subjects significantly increased from 21 to 46 μU/ml at the completion of the infusion and rapidly declined. In the three obese subjects restudied after 3- and 24-day fasts an approximate twofold increase of serum insulin was observed after each acetoacetate infusion.

The mean fractional utilization rate of exogenously derived ketone bodies for all 12 subjects after an overnight fast was 2.9% min^{-1}. In the three obese subjects studied after an overnight, 3 and 24 day fast the mean fractional utilization rates were 2.1%, 1.5%, and 0.6% min^{-1}, respectively. Ketone body volumes of distribution in the overnight fasted subjected varied from about 18% to 31% of body wt, suggesting that ketone bodies are not homogenously distributed in the body water. In the three obese subjects restudied after 3- and 24-day fasts volumes of distribution remained approximately constant. When total ketone body concentrations in the blood were below 2.0 mmol/liter, there was a linear relationship between ketone body utilization rates and ketone body concentrations; no correlation was found when blood concentrations were higher.

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INTRODUCTION

The cardinal role of insulin in regulating the release of free fatty acids (FFA) [1] from adipose tissue into the blood stream is well recognized. The decline in circulating insulin during starvation is thought to initiate lipolysis (1, 2). Hepatic production of ketone bodies is due, at least in part, to the rate of influx of FFA to the liver (3). During starvation the initial rise in ketone bodies, acetoacetate (AcAc) and β-hydroxybutyrate (β-OHB), reflects an imbalance between production and removal rates. However, as starvation progresses blood AcAc and β-OHB plateau at elevated concentrations. After 24 days of starvation the production rates of AcAc and

[1] *Abbreviations used in this paper:* AcAc, acetoacetate; FFA, free fatty acids; IRI, immunoreactive insulin; β-OHB, β-hydroxybutyrate.

TABLE I
Clinical Data

Subject	Age	Sex	Height	Fasting weight			Deviation from pop. mean wt.*			IVGTT‡	Diagnoses
				Overnight	3 day	24 day	Overnight	3 day	24 day		
	yr		*cm*	*kg*			*%*			K_g	
P. O.	33	F	161	52			88			1.70	Normal
J. M.	25	M	179	75			100			2.42	Normal
G. R.	39	M	173	69			94			2.49	Normal
G. B.	35	M	183	79			98			1.33	Normal
M. S.	28	M	171	67			96			1.84	Normal
L. G.	41	M	188	96			104			2.00	Normal
S. D.§	26	F	163	63			110			—	Normal
R. M.	22	F	160	68			127			1.91	Normal
J. McC.	43	M	180	103			128			0.74	Obesity; diabetes, chemical
K. R.	21	F	183	110	107	97	158	152	138	1.03	Obesity; hypertension
G. S.	46	F	157	124	120	109	205	199	180	1.07	Obesity
L. J.	57	F	163	140	137	125	215	209	191	0.94	Obesity; congestive heart failure; diabetes, chemical

* From Metropolitan Life Insurance tables, 1959.
‡ Intravenous glucose tolerance test (0.5 g/kg). Values expressed as absolute percent disappearance per minute.
§ S.D. had a normal 2 h postprandial blood glucose concentration.

β-OHB are in equilibrium with the removal rates and a new steady state is accomplished (4).

A feedback cycle has been suggested whereby ketone bodies contribute to the maintenance of this new steady state of hyperketonemia by modulating insulin secretion, thereby regulating lipolysis and preventing fatal ketoacidosis (5). The insulinogenic role of ketone bodies in man, however, has been controversial (6–10) and has not been documented in starvation-induced hyperketonemia. Furthermore, another feedback cycle not mediated by insulin has been proposed whereby ketone bodies directly inhibit the release of FFA from adipose tissue (11–14).

This study was designed to assess the influence of ketone bodies on insulin and/or FFA release in response to rapid intravenous administration of NaAcAc. The results were also used to determine the disappearance rates of AcAc and β-OHB from the blood, to calculate the volume of ketone-body distribution, and to estimate rates of tissue uptake of ketone bodies in man during progressive starvation.

METHODS

Subjects. 12 adult volunteers of both sexes weighing between 88 and 215% of ideal body weight were admitted to the General Clinical Research Center of Temple University Hospital (Table I). Each subject was informed of the procedures planned and the potential risks involved in administering intravenous NaAcAc. All volunteers had normal serum thyroxine levels and normal renal and hepatic functions. Intravenous glucose tolerance tests were performed as previously described (4) in all subjects except S. D. who had a normal 2 h postprandial blood glucose concentration. Three of the volunteers (K. R., G. S., and L. J.) were obese and underwent therapeutic fasting for weight reduction. The possible risks involved with starvation were explained to these subjects before fasting. One subject (L. J.) presented with mild congestive heart failure which compensated during starvation without using cardiotonic or diuretic drugs. The three obese subjects who underwent starvation had a daily intake of one multivitamin capsule (Unicap, Upjohn Co., Kalamazoo, Mich.), 17 mEq of NaCl (sugar-free tablets), 1,500 ml of water and, as tolerated, 13 mEq of KCl (gelatin capsules).

Infusion procedure and chemical analyses. Studies were performed in all subjects after an overnight fast. In three obese subjects (K. R., G. S., and L. J.) studies were repeated after 3 and 24 days of total starvation.

Indwelling venous cannulae were inserted in both antecubital veins and maintained patent with a slow infusion of saline. One venous route was used to infuse NaAcAc, approximately 1.0 mmol/kg body wt, while the other venous route was used to obtain blood samples for determination of serum immunoreactive insulin (IRI), blood AcAc, β-OHB, glucose, and double-extracted plasma FFA (15) at 10 min and immediately before the 5 min period of NaAcAc infusion, at 2½ and 5 min during the infusion, and at 7½, 10, 15, 20, 30, 40, 50, 60, 90, and 120 min postinfusion. The overnight fasted volunteers voided immediately before the infusion and again at the end of the 2 h sampling period. Urine specimens were collected from the three obese subjects who underwent prolonged starvation on days 3 and 24 for 2 h before the NaAcAc infusion and again at the end of the 2 h study in order to assess the effect of NaAcAc infusion on base line urinary ketone body excretion. Urine volumes were measured and aliquots were analyzed for AcAc and β-OHB. AcAc in blood and urine was determined on the same day of the study. All substrate and hormone determinations were done in duplicate.

Calculation of ketone-body disappearance rates (K), volume of distribution, pool size, and rates of tissue uptake.

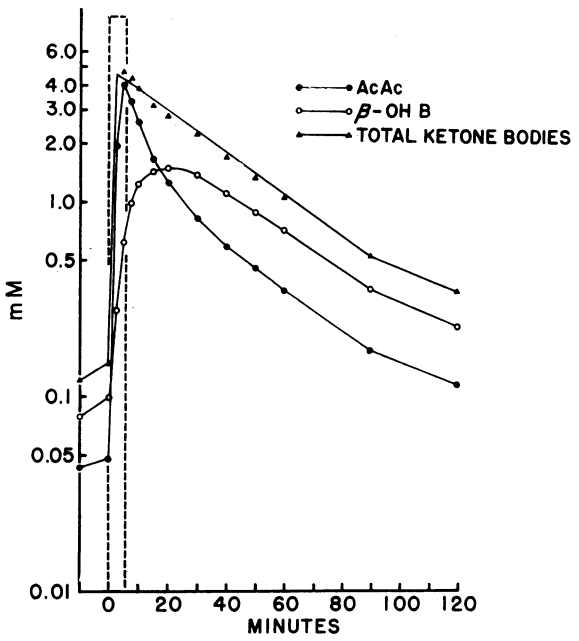

FIGURE 1 Circulating ketone-body responses to rapid intravenous NaAcAc infusion in man after an overnight fast. The millimolar concentrations of AcAc, β-OHB, and total ketone bodies are plotted semilogarithmically versus time. The infusion period is indicated by the dashed bar.

Fractional disappearance rate constants (K) for excess AcAc, β-OHB, and total ketone bodies[2] were calculated by graphical analysis of semilogarithmic plots of their respective blood concentrations as described by Amatuzio et al. (16). Best straight line fits of the experimental values obtained from 30–90 min were calculated by least squares analysis for AcAc, β-OHB, and total ketone bodies. The slopes of these lines were used to calculate the K values for AcAc, β-OHB, and total ketone bodies by the usual methods (17). The 30–90 min period was chosen because blood AcAc and β-OHB levels declined exponentially in all subjects during this interval. Preceding this interval, a mixing phase of the administered AcAc was obvious and the levels of β-OHB had not always begun to decline. During the 90–120 min period after an overnight fast, the AcAc and β-OHB blood levels approached their base line values in an asymptotic manner. Extrapolation of the 30–90 min portion of the excess total ketone-body curves to time zero postinfusion (5 min interval) was done to estimate the initial total ketone body concentration due to the administered NaAcAc in order to calculate its volume of distribution.

The volume of distribution (V_{1liter}) was operationally defined and estimated from the equation: $V_{1liters}$ = Amount injected (mmol)/(extrapolated time zero concentration (mmol/liter)), and expressed in liters and as percent of body wt. The pool (P) was calculated from the equation $P = V_{1liters} \times$ base line concentration (mmol/liter) and expressed in millimoles. Rates of tissue ketone-body utilization (K_u) were calculated as the difference between the rates of total ketone-body disappearance rates (K_d) minus rates of total ketone-body urinary excretion rates (K_e). The product of $K_u \times P \times 1{,}440$ (min/24 h) was equated to tissue utilization in mmol or g/24 h.

Statistical analyses. The effects of rapid intravenous administration of NaAcAc on circulating concentrations of FFA, insulin, and glucose were analyzed for statistical significance by the paired t test for small sample numbers (18). Correlation coefficients were calculated as described by Snedecor and Cochran (19). Values are given as mean ±SEM (19).

Preparation of sodium acetoacetate. A modification of the method of Krebs and Eggleston was employed (20). To a known quantity of fresh, triple-distilled reagent grade ethyl acetoacetate (Fisher Scientific Co., Pittsburgh, Pa.) a slight excess of 2 N NaOH was added. The mixture was incubated 1½ h at 40°C, and then placed in ice-bath and neutralized (pH 7.0) with 1 N HCl. Hydrolysis was about 98% completed on the basis of titration and about 94% completed on the basis of enzymatic assay. The reaction mixture was lyophilized to approximately 1/4 the original volume. No ethanol was detectable (21). The NaAcAc solution was sequentially filtered through a 1.2 μm and a 0.22 μm

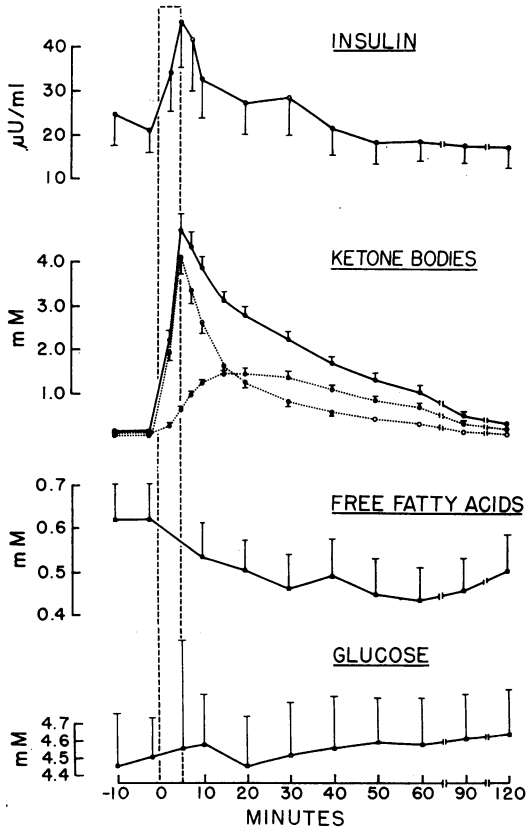

FIGURE 2 Temporal relationships of circulating immunoreactive insulin and substrate responses to rapid intravenous NaAcAc infusion in man, mean ±SEM. The infusion time is indicated by the dashed bar. In the ketone-body panel at the 10 min postinfusion time interval the solid top line is total ketone bodies, the middle dotted line is AcAc and the bottom dotted line is β-OHB.

[2] AcAc, β-OHB, and total ketone-body excesses are defined as the amounts of AcAc, β-OHB, and total ketone bodies in mmol/liter in excess of base line values for the compounds before infusing NaAcAc.

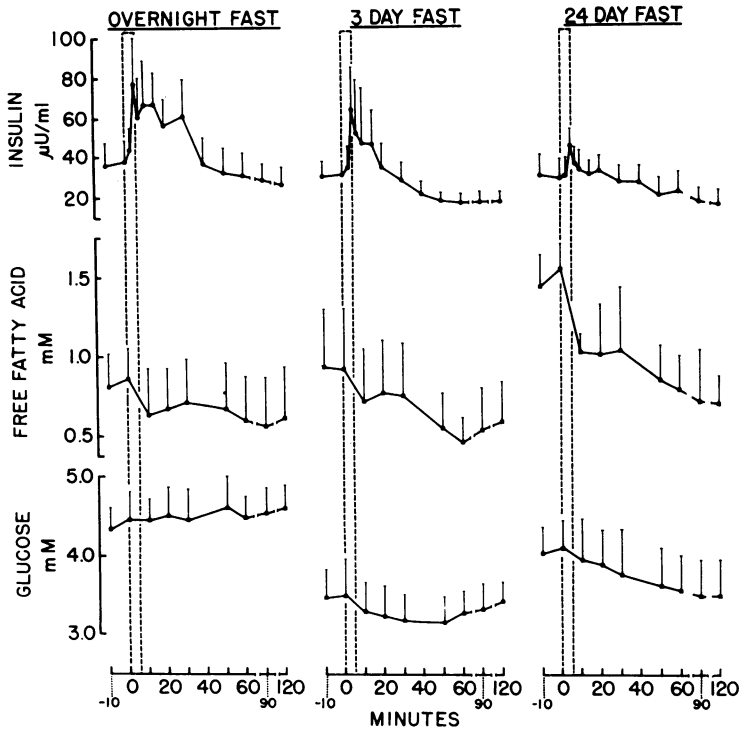

FIGURE 3 Circulating immunoreactive insulin, free fatty acids, and glucose responses to rapid NaAcAc infusion in three obese subjects after overnight and 3 and 24 days of starvation. The infusion time is indicated by the dashed bar.

Swinnex filter unit (Millipore Corp., Bedford, Mass.) and stored at −20°C until used. Sterility of the solution was established by the absence of bacterial growth after 72 h in appropriate culture media. Before infusion the preparations were reassessed for AcAc concentration.

RESULTS

Fig. 1 shows the mean blood ketone-body responses to rapid intravenous NaAcAc infusion in all overnight fasted subjects. The millimolar concentrations of AcAc, β-OHB, and total ketone bodies were plotted semilogarithmically against time. AcAc concentrations rose rapidly, reaching a peak concentration at the end of the infusion period. β-OHB concentrations also increased rapidly and exceeded those of AcAc 10 min after completion of the infusion. The total ketone body concentration at the end of the infusion period was 4.74±0.39 mmol/liter, a level comparable to that found after prolonged starvation (4). After the initial mixing period AcAc, β-OHB, and total ketone bodies declined in a parallel manner. There were no obvious differences between the lean and obese subjects with regard to their blood concentrations of ketone bodies.

Fig. 2 is a composite figure showing the temporal relationships of insulin and substrate responses to AcAc infusion after an overnight fast. FFA concentrations were significantly depressed ($P < 0.05$) from the 20th to 90th min postinfusion period. The base line FFA concentration of 0.61±0.10 mmol/liter reached a nadir of 0.43±0.11 mmol/liter at 60 min ($P < 0.03$). There was no change in blood glucose.

In all 12 subjects the mean ±SEM base line IRI concentration of 21±5 μU/ml rose to 46±11 μU/ml ($P < 0.03$) at the completion of the infusion and declined rapidly thereafter. Both the basal IRI concentrations and the increases above basal concentrations were greater in obese subjects than in lean subjects. In addition there was a good correlation between the peak IRI response to infused NaAcAc and the ponderal index ($r = 0.88$; $P < 0.001$) (22).

To assess the insulin response to ketone bodies and the disappearance of ketone bodies during fasting, NaAcAc administration was repeated after 3 and 24 days of starvation in three obese subjects (K. R., G. S., and L. J.). The characteristic fall in the blood glucose and serum insulin with the subsequent elevation of plasma FFA and blood ketone bodies known to occur with fasting was observed in the three obese subjects (1, 4).

Fig. 3 demonstrates that the insulin, FFA, and glucose responses to infused AcAc after an overnight fast persisted after 3 and 24 days of fasting. The mean preinfusion IRI concentrations after overnight and 3- and

TABLE II
Rapid Intravenous Sodium

Subjects	NaAcAc administered	Urinary ketone bodies		K_d			K_u
				AcAc	β-OHB	Total	Corrected*
	mmol	*mmol/2 h*	*%/2 h*		*% min⁻¹*		*% min⁻¹*
Overnight fast							
P. O.	55.1	3.49	6.3	3.7	4.2	4.02	3.97
J. M.	80.5	5.17	6.4	3.7	3.7	3.70	3.65
G. R.	74.4	2.41	3.2	3.7	3.9	3.79	3.76
G. B.	78.2	3.68	4.7	3.5	3.4	3.42	3.38
M. S.	74.5	5.03	6.8	3.1	2.5	2.67	2.61
L. G.	87.7	3.89	4.4	4.6	3.3	3.68	3.64
S. D.	64.8	6.21	9.6	—	—	—	—
R. M.	66.3	8.06	12.2	3.4	3.0	3.11	3.01
J. McC.	155.7	6.15	5.3	2.4	1.9	2.06	2.02
K. R.	127.6	12.92	10.1	3.8	3.6	3.19	3.11
G. S.	129.1	2.07	1.6	2.7	2.4	1.34	1.33
L. J.	145.6	6.83	4.7	2.4	2.2	1.93	1.89
Grand mean	95.0	5.49	6.3	3.4	3.1	2.99	2.94
±SEM	±10.0	±.85	±.9	±0.2	±0.2	±0.27	±0.26
Mean±SEM (K. R., G. S. and L. J.)	134.1 ±5.8	7.27 ±3.14	5.5 ±2.5	3.0 ±0.4	2.7 ±0.4	2.15 ±0.55	2.11 ±0.53
3 day fast							
K. R.	100.2	34.77	34.7	1.7	1.20	1.39	1.10
G. S.	132.6	41.47	31.3	1.6	0.07	1.19	0.93
L. J.	137.3	8.65	6.3	2.7	2.60	2.46	2.41
Mean	123.4	28.30	24.1	2.0	1.29	1.68	1.48
±SEM	±11.7	±10.01	±9.0	±0.3	±0.73	±0.39	±0.47
24 day fast							
K. R.	92.4	49.24	53.3	2.1	0.16	0.94	0.50
G. S.	97.8	46.29	47.3	1.7	0.14	0.87	0.48
L. J.	133.1	20.80	15.6	1.4	0.71	1.03	0.90
Mean	107.8	38.78	38.7	1.7	0.34	0.95	0.63
±SEM	±12.8	±9.03	±11.7	±0.2	±0.19	±0.05	±0.14

* Total blood ketone bodies K_d (% min⁻¹) minus total urinary ketone bodies K_e (% min⁻¹).

‡ Extrapolated ketone-body concentration at the time NaAcAc infusion was completed. Since mixing was not complete at this time the actual measured blood concentrations exceeded those listed.

24-day fasts were 38, 31, and 29 μU/ml, respectively. After the overnight and 3- and 24-day fasts the time of individual maximal IRI responses to NaAcAc infusions varied from 5 to 30 min. The mean maximal increase over base line concentrations were 2.2-, 2.1-, and 1.7-fold, respectively. Because of the time variation the mean peak value shown in Fig. 3 does not reflect the individual maximal increases observed in IRI after NaAcAc infusions. Subsequent to each NaAcAc infusion FFA concentrations declined. Overnight fasting plasma basal FFA concentration of 0.83±0.23 mmol/liter decreased at 90 min to 0.55±0.30 mmol/liter; 3 day fasting basal concentration of 0.92±0.38 mmol/liter decreased at 60 min to 0.46±0.15 mmol/liter and 24 day fasting basal concentration of 1.49±0.18 mmol/liter decreased at 120 min to 0.71±0.33 mmol/liter. After the infusion in each subject the decreases in plasma FFA that occurred exceeded the methodological errors (15). The overnight fasted obese subjects had a mean blood glucose concentration of 4.39±0.31 mmol/liter and remained relatively constant. After the 3 day fast base line concentrations of 3.49±0.41 mmol/liter declined to a low of 3.22±0.32 mmol/liter 50 min postinfusion and returned to base line values 2 h postinfusion. After the 24 day fast base line concentrations of 4.07±0.34 mmol/liter progressively decreased to 3.49±0.45 mmol/liter

Acetoacetate Infusion Study

Ketone bodies				Concentration			
				Preinfusion			Extrapolated (time zero)‡
Volume of distribution		Pool	Tissue utilization rate	AcAc	β-OHB	Total	Total
liters	*% body wt*	*mmol*	*mmol/24 h*			*mM*	
12.6	24.1	2.39	137	0.08	0.11	0.19	4.39
18.1	24.2	0.91	48	0.02	0.03	0.05	4.44
16.3	23.6	1.30	71	0.04	0.04	0.08	4.56
19.6	24.7	1.57	76	0.04	0.04	0.08	3.99
17.7	26.5	1.59	60	0.02	0.07	0.09	4.20
19.5	20.3	3.35	176	0.06	0.11	0.17	4.49
—	—	—	—	0.04	0.16	0.20	—
18.4	27.0	1.69	73	0.02	0.07	0.09	3.60
31.8	30.7	5.47	159	0.06	0.11	0.17	3.64
21.5	19.5	2.67	119	0.04	0.09	0.13	5.93
34.1	27.4	6.72	129	0.06	0.13	0.19	3.79
26.0	18.5	4.84	132	0.07	0.12	0.19	5.59
21.4	24.2	2.95	107	0.05	0.09	0.14	4.42
±2.0	±1.1	±.58	±13	±0.01	±0.01	±0.02	±0.23
27.2	21.8	4.74	127	0.06	0.11	0.17	5.10
±3.7	±2.8	±1.17	±4	±0.01	±0.01	±0.02	±.66
22.0	20.6	74.58	1181	0.85	2.54	3.39	4.56
27.4	23.1	55.40	742	0.59	1.41	2.00	4.79
20.4	14.9	12.44	432	0.21	0.40	0.61	6.74
23.3	19.5	47.47	785	0.55	1.45	2.00	5.36
±2.1	±2.4	±18.37	±217	±0.19	±0.62	±0.80	±0.69
22.6	23.3	148.48	1,069	1.32	5.25	6.57	4.09
19.8	18.1	160.97	1,112	2.01	6.12	8.13	4.95
17.0	13.7	119.85	1,553	1.25	5.80	7.05	7.81
19.8	18.4	143.10	1,245	1.53	5.72	7.25	5.62
±1.6	±2.8	±12.17	±155	±0.24	±2.54	±0.46	±1.12

at the end of the 2 h study period. The decreases in blood glucose observed in each subject after the 3 and 24 day NaAcAc infusions exceeded the methodological errors (15).

Fig. 4 shows the effects of progressive starvation on the blood ketone body responses to NaAcAc given intravenously to subjects K. R., G. S., and L. J. After NaAcAc infusion the incremental increases in the mean blood AcAc concentration were comparable, being 5.11, 5.52, and 5.43 mmol/liter after the overnight and 3- and 24-day fasting periods, respectively. Increases in blood β-OHB concentrations were comparable after each fasting period. The curves in the left panel of Fig. 4 display rapid removal of exogenous AcAc with a small portion appearing as blood β-OHB which also rapidly disappeared. The curves in the middle panel reveal the results after a 3 day fast. They differ from the overnight findings. AcAc disappeared from the blood at a slower rate. Also a marked decrease in β-OHB disappearance occurred. The curves in the right panel show the results after the 24 day fast. At this time the slowest removal of exogenous AcAc occurred; after the maximum blood β-OHB concentration developed, it remained approximately constant through the remainder of the 2 h observation period. In essence, as starvation progressed, there were diminished removal rates of exogenously de-

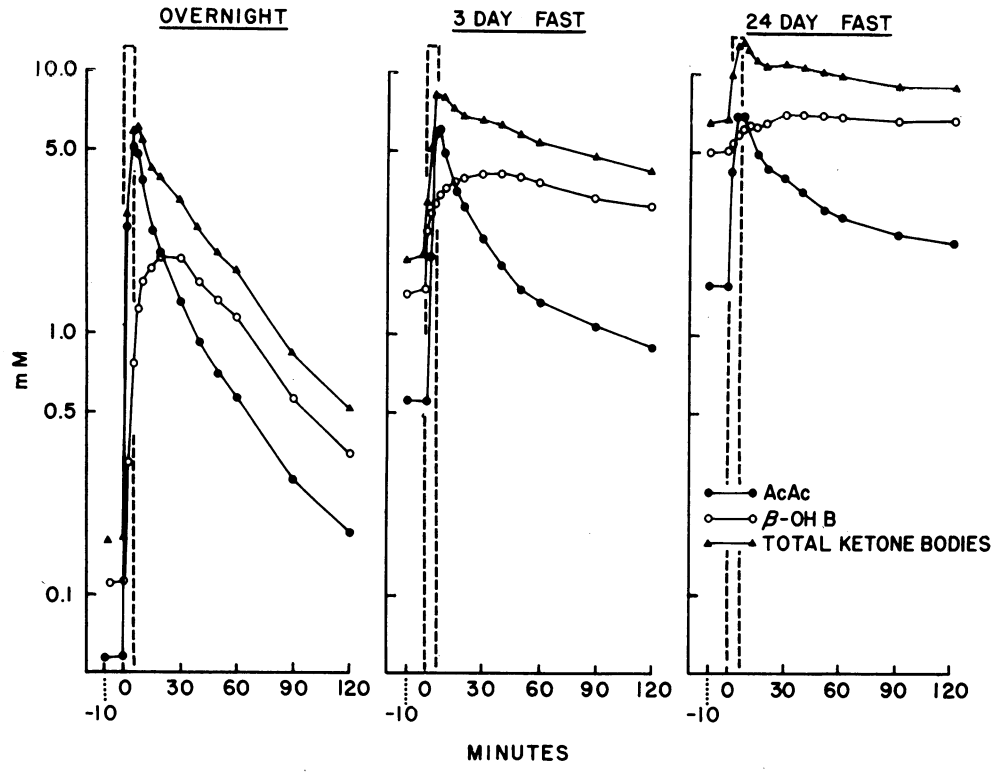

FIGURE 4 Circulating ketone-body responses to rapid intravenous NaAcAc infusion in three obese subjects after overnight and 3 and 24 days of starvation. AcAc, β-OHB, and total ketone bodies are plotted semilogarithmically versus time. The infusion period is indicated by the dashed bar.

rived AcAc, β-OHB and, hence, total ketone bodies, and there was a longer time required to achieve maximum increases in blood β-OHB concentrations.

Table II shows the quantities of NaAcAc administered, urinary losses, preinfusion blood ketone body concentrations, extrapolated time zero total ketone-body concentrations, ketone-body disappearance rates (K_d), tissue utilization rates (K_u), volumes of distribution, pools, and estimated daily tissue utilization rates. Preinfusion ketone body concentrations after the overnight fast were comparable to those previously reported (4, 15). The exogenously derived ketone bodies rapidly disappeared from the blood stream as reflected by a mean total ketone-body K_d of 3.0% min^{-1} for all subjects and a comparable mean value of 2.2% min^{-1} for the three obese subjects (K. R., G. S., and L. J.). The mean urinary ketone body excretion for all subjects was only 6.3% of the injected dose and for the three obese subjects was 5.5% of the administered load. Thus, the calculated rate constants (K_d) for the disappearance of AcAc, β-OHB, and total ketone bodies from the blood during the overnight fast study are not significantly influenced by ketonuria and reflect reasonable estimates of tissue uptake (K_u). However, when the three obese subjects were restudied after 3- and 24-day fasts the mean urinary ketone-body excretion was 24.1 and 38.8%, respectively, of the exogenous AcAc load. These values are corrected for the basal ketonuria that occurred at 3 and 24 days. Not only was more (if not most, as in the case of K. R.) of the administered AcAc load excreted in the urine as starvation progressed, but also the fractional disappearance (K_d) of AcAc, β-OHB, and total ketone bodies progressively decreased with starvation (Fig. 4 and Table II). Because of the large urinary loss of ketone bodies calculated disappearance rates for 3- and 24-day studies must be corrected for ketonuria if they are to reflect tissue utilization rates (K_u).

The calculated volume of distribution for ketone bodies vary from 18.5 to 30.7% body weight in the overnight fasted subjects. These volumes of distribution suggest that ketone bodies are not homogenously distributed throughout various water spaces of different tissues, since they exceed those reported for extracellular fluid volume but are less than those reported for total body fluid volume (23, 24). Similar incremental increases in blood ketone bodies were observed after over-

night and 3- and 24- day fasts. This is in accord with our observations that the volumes of distribution remained constant throughout the starvation period.

Although animal studies have shown concentration differences for ketone bodies between blood and various tissues (25–27), recent investigations have shown that for the bulk of the body the ketone body gradient between intracellular and extracellular water is constant (27). Since ketone bodies are not uniformly distributed in the body, actual volumes of distribution cannot be obtained by extrapolating the blood ketone body disappearance curves to time zero and calculating volumes of distribution. Such procedures underestimate the true fluid volumes in which ketone bodies are dispersed. Nevertheless, operational volumes of distribution for ketone bodies can be calculated from the disappearance curves, and thus reasonable ketone body pools can be estimated.

There were no significant differences among the rates of disappearance (K_d) of ketone bodies from the bloodstream of these lean and obese subjects. There was a progressive decrease in the mean K_d or K_u in the obese subjects as starvation was prolonged. However, due to the higher blood ketone-body concentrations, and thus the larger ketone-body pools, the rates of tissue utilization increased as starvation progressed (overnight vs. 24 day, $P < 0.05$).

The relationship between blood ketone body concentration and utilization rate (K_u) is shown in Fig. 5. In general there was an increase in the ketone body utilization rate as the blood concentration increased ($r = 0.91$, $P < 0.001$). The insert in Fig. 5 depicts the sharp rise in utilization as the concentration increased from 0.05 to 0.60 mmol/liter ($r = 0.98$, $P < 0.001$). However, at blood ketone-body concentrations between 2.00 and 8.13 mmol/liter there is no correlation between utilization rates and blood concentrations ($r = 0.59$, $P < 0.3$).

DISCUSSION

Metabolic effects of rapidly administered NaAcAc. Ketone bodies are known to be insulinogenic in dogs (5) and rats (14). Previous studies on the insulinogenic effect of these substrates in man have been controversial (6–10). The present study shows that ketone bodies are capable of stimulating insulin secretion in man. The insulin response to infused AcAc was great enough to be manifested by a rise in peripheral serum insulin concentrations. The previously observed differences in human studies can be explained, at least in part, by the temporal

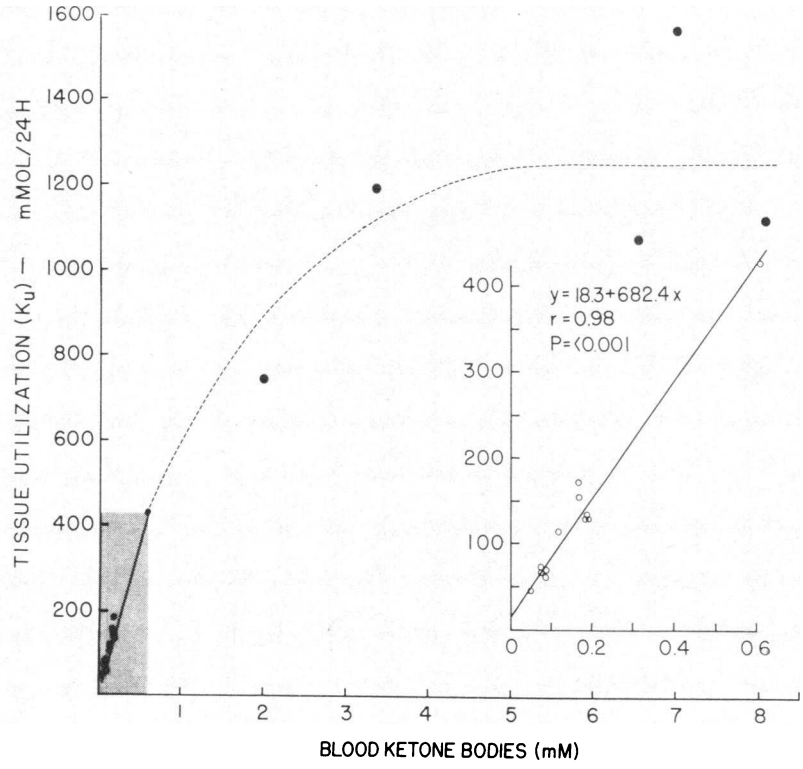

FIGURE 5 The relation between circulating ketone-body concentrations and the rates of tissue utilization. For low concentrations see the insert.

relationship between the administration of ketone bodies and blood sampling time, and by the presence or absence of obesity in the subjects. In this study the peripheral insulin concentration reached a mean peak at the end of the 5 min NaAcAc infusion period and then rapidly declined. Furthermore, the insulin response to NaAcAc was potentiated by obesity. This latter finding is similar to the observation of others who have demonstrated that obesity is accompanied by beta-cell hyperresponsiveness to numerous secretogogues (28, 29). The insulin response to infused AcAc observed after an overnight fast could also be demonstrated in the obese subjects studied after 3 and 24 days of fasting. After each infusion period there was an approximate twofold increase in the maximum peripheral insulin concentration. The fall in plasma FFA coincident with the AcAc-stimulated insulin release is consistent with the well recognized antilipolytic action of this hormone. From the data presented here it appears that ketone bodies can lower the concentration of circulating FFA by stimulating insulin release. Evidence from the work of others suggests that ketone bodies have a direct antilipolytic action (11–14). This possibility can not be established from the data presented here.

It has been previously demonstrated that the administration of ketone bodies to man produces a decrease in blood glucose as well as plasma FFA (6–8, 10, 30). We observed no change in blood glucose after an overnight fast. While methodological differences may account for these divergent observations, other reasons may be suggested. Previous studies have shown that ketone bodies effectively compete with FFA and glucose as body fuels for oxidative metabolism (31–34). In this study it was shown that rapid intravenous infusion of NaAcAc caused a significant rise in mean serum insulin concentration (Fig. 2). After an overnight fast insulin apparently diminishes hepatic release of glucose (35). Therefore AcAc may modify both peripheral oxidation of glucose and hepatic release of glucose into the circulation. The stability of blood glucose concentration after the overnight fasting study suggests that diminished hepatic glucose release was equaled by diminished peripheral glucose uptake. In the three obese subjects studied after 3- and 24-day fasts, base line blood glucose concentrations fell after NaAcAc infusion. The decline in blood glucose concentration in the obese subjects at 3 and 24 days of fasting may have been due to either diminished hepatic glucose release (35) or increased peripheral glucose uptake (36).

Kinetics of intravenous NaAcAc infusion. Studies of ketone-body metabolism are complicated because of variations in the rates of production and utilization between AcAc and β-OHB (4, 15, 37). However, these substrates are readily interconverted by tissue 3-hydroxybutyrate dehydrogenase, and the β-OHB formed after AcAc infusion must be reconverted to AcAc before oxidation (3). For simplification, the disappearance of total ketone bodies (AcAc plus β-OHB) from blood and excretion of total ketone bodies in urine were used empirically to calculate volumes of distribution, pools, and rates of tissue uptake during different stages of starvation.

The volume of distribution of ketone bodies, calculated by extrapolation of the disappearance phenomenon, is larger than the extracellular fluid volume and smaller than the total body fluid volume (23, 24). The failure to correlate ketone body volumes of distribution with anatomical spaces (volumes) implies that these substrates are not homogenously distributed in the various water compartments of the body. Thus, our human studies support the experimental findings observed in tissue fragments (25–27) and in whole animals (38). However, the operational volume of distribution calculated here for ketone bodies is equal to that fluid volume in which these substrates would be dispersed if their concentrations were homogenous. Therefore, an operational volume of distribution can be defined and a pool calculated.

The operational volumes of distribution are not dependent upon the disappearance rates of total ketone bodies from the bloodstream. This conclusion is drawn from the data showing the total ketone body volume of distribution, determined after the bloodstream concentrations were acutely elevated and expressed as percent of body wt., remained at about 20% in the three subjects studied after an overnight, 3 and 24 day fast while the K_4 rate decreased from 2.2% min^{-1} to 1.0% min^{-1}. Thus, no apparent expansion of available space for ketone bodies occurred subseqent to starvation, although the rates of disappearance from the blood were significantly decreased.

The fate of the intravenously administered AcAc was not definitely determined. However, it is obvious that the disappearance of exogenously administered AcAc from the circulation depends on its mixing in the extracellular fluids, on its cellular penetration and oxidation, on its reduction to β-OHB, and on renal excretion of AcAc and β-OHB. Since starvation is a catabolic state, it is unlikely that much of the acetyl-CoA derived from ketone body cleavage was used for net synthesis, i.e., lipogenesis (39). It is more reasonable to assume that the ketone bodies that disappeared from the blood and escaped renal excretion were oxidized to CO_2 and water. The loss of ketone bodies in the urine was not an important factor in the disappearance of ketone bodies from the circulation after an overnight fast (Table II). However, as starvation progressed, ketonuria became increasingly important as a route of exit for the exogenously derived blood ketone bodies. This was exemplified by one of our obese subjects (K. R.). After a 24

day fast her renal excretion could account for about one-half of the exogenously derived ketone bodies that disappeared from the blood. Heightened ketonuria after the NaAcAc infusion was probably due to the fact that other routes of disposal were saturated. This does not imply that the body oxidized less ketone bodies as starvation progressed, but does indicate that ketonuria can be an important mechanism for man to dissipate the additional ketone-body loads.

Employing the catheterization technique coupled with regional blood flow, hepatic ketone body production rates have been estimated to be 9–45 g/24 h in man after an overnight fast with venous blood ketone body concentrations of 0.1–0.4 mmol/liter (40, 41). After a 3 day fast hepatic production rates have been estimated to be 115–125 g/24 h with the venous blood ketone body concentration of about 3.0 mmol/liter (42).[3,4] After a 3–6 wk fast when circulating concentrations of ketone bodies increase to about 7.0 mmol/liter, net ketone-body production and utilization have been estimated to be about 92–112 g/24 h (4, 15). Fasting blood ketone-body concentrations are relatively constant on the hour to hour basis. Under such steady-state conditions the rates of utilization are equal to rates of production. The mean rate of utilization, and therefore, the mean rate of production, obtained from rapid intravenous NaAcAc administration after an overnight, 3 and 24 day fast were about 13, 80, and 128 g/24 h, respectively.[5] Quantitatively, these values are in agreement with previously reported ketone body production rates estimated by catheterization techniques.

Maximum rates of ketone-body production and utilization are probably achieved during starvation when the concentration of total ketone bodies has increased to about 2–3 mmol/liter. Under conditions of maximum tissue utilization almost complete recovery of the administered NaAcAc in urine might be anticipated. Hence, the disappearance rate of ketone bodies from the blood would only reflect renal clearance. However, after 3 and 24 days of starvation urinary excretion accounted for about 24 and 39%, respectively, of the administered AcAc load. Rates of tissue uptake (Table II), which were calculated after correction for urinary excretion, are in good agreement with those obtained by other methods. This suggests that the administration of NaAcAc, which was insulinogenic, either promptly suppressed hepatic production or promoted peripheral ketone-body utilization (42).

In this study the rates of utilization (production) were directly related to blood ketone body concentrations up to about 0.6 mmol/liter and perhaps up to 2–3 mmol/liter. At higher concentrations, circulating levels did not influence utilization or reflect production to the same degree. Our human data are in general agreement with those obtained from rabbits (43), dogs (44), and rats (45, 46).

It appears in man that the utilization of ketone bodies increase with rising blood concentrations until a maximum rate of about 128 g/24 h is reached; thereafter heightened ketonemia and ketonuria occur with little, if any, increase in tissue utilization.

[3] Personal communication from Dr. R. J. Havel, University of California, San Francisco, Calif.

[4] Garber, A. J., P. H. Menzel, and O. E. Owen. 1973. Ketogenesis and gluconeogenesis in humans. Submitted for publication.

[5] The average molecular weight of AcAc and β-OHB is 103. Millimoles/24 h can be converted to g/24 h by multiplying mmol/24 h × 103 and dividing by 1,000.

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