Ketone Body Production and Disposal in Diabetic Ketosis

A Comparison with Fasting Ketosis

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SUMMARY

This work compares the metabolism of total ketone bodies in 13 insulin-deprived, type I diabetic subjects and 26 control subjects fasted for 15 h to 23 days, with the two groups showing a similar range of ketone body levels (1–12 mM). Ketone turnover rate was measured using a primed, constant infusion of either ^{14}C-acetoacetate or ^{14}C-β-hydroxybutyrate, both tracers yielding comparable results.

The major conclusions of this study are the following: the kinetics of ketone bodies are comparable in the two groups within the range of concentrations tested. The hyperketonemia of fasting and diabetes is primarily caused by an increased production of ketone bodies, but the phenomenon is amplified by a progressive limitation in the ability of tissues to remove ketones from blood as the concentration rises. The inverse relationship between the metabolic clearance and the plasma levels of ketones, which underlies this process, represents a general characteristic of ketone body metabolism that applies to both types of ketosis. A maximal metabolic disposal rate of about 2.3 mmol/min/1.73 m^2 is attained in both groups at concentrations of 10–12 mM, which correspond to the highest ketone body levels encountered during prolonged fasting. Thus, up to these levels, there is no evidence for the existence of a ketone body removal defect specific to diabetes. DIABETES 1985; 34:326–32.

Only a few studies have been devoted to the measurement of the rate of transport of ketone bodies (KB) in diabetic man,[1,2] and the important question of the relative contribution of overproduction and underutilization in the genesis of hyperketonemia has not hitherto received an unequivocal answer. It is not known, either, whether significant differences exist in the kinetics of KB between diabetic and fasting ketosis at various KB levels. The limitations of our knowledge in this field can be explained by several factors, including the variability in the techniques used for measuring KB transport, the small number of subjects involved in most individual studies, and the frequent use of grossly overweight subjects in studies on fasting.

The present work provides measurements of KB turnover rates in insulin-dependent diabetic subjects and in normal, nonobese fasted subjects, the design of the study allowing for a close comparison between the two groups over a wide range of KB levels.

MATERIALS AND METHODS

Diabetic subjects. Over a 12-year period, 13 type I diabetic subjects volunteered for participation in the study.

The first 10 subjects listed in Table 1 had a known history of diabetes and were hospitalized for the purpose of the study. They were deprived of s.c. insulin 36–96 h before the experiments. After s.c. insulin withdrawal, seven of these patients (nos. 1, 4, and 6–10) were placed on i.v. insulin to prevent gross hyperglycemia and ketonuria, and the insulin infusion was discontinued 3–8 h before the study. Subjects no. 2, 3, and 5 were less insulin-dependent and did not need i.v. insulin during the interval between the last s.c. injection and the experiment. Subject no. 11 was a noncompliant patient who was studied 40 days after he had ceased taking insulin. Finally, patients no. 12 and 13 had no history of diabetes and were hospitalized for a diabetic decompensation that was the first clinical manifestation of their disease. All patients were alert and in good nutritional status. They were moderately hyperglycemic (Table 1) and had no clinical signs of dehydration. In the most ketotic patient (no. 13), arterial blood pH was 7.32 and plasma total CO_2 concentration was 11 mM.

Control subjects. The main characteristics of the 26 control subjects are presented in Table 1. Their relative body weights and ages were comparable to those of the diabetic subjects. The duration of the fast before the study varied between 15 h (overnight fast) and 23 days (mean 3.9 days) so

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TABLE 1
Characteristics of diabetic and control subjects

	Age (yr)	Sex	% Ideal body weight	Body surface (m^2)	Glucose (mg/dl)	ACAC (mM)	β-OHB (mM)	ACAC + β-OHB (mM)	FFA (mM)	Tracer used†	History of diabetes (yr)	Insulin: Usual dose (U/day)	Insulin: Time since last s.c. injection (h)	Insulin: Time since end of i.v. infusion (h)
Diabetic subjects (N = 13)														
1*	37	M	107	2.00	230	0.25	0.66	0.91	0.90	B	8	56	48	8
2	49	F	108	1.69	277	0.34	1.73	2.07	0.80	A	26	28	60	—
3	30	F	88	1.45	299	0.91	2.73	3.64	0.78	A	15	70	96	—
4*	46	M	107	1.80	292	0.69	2.47	3.15	1.09	B	14	60	48	4
5	26	F	124	1.75	268	1.12	2.98	4.10	1.00	A	6	55	48	—
6*	29	F	115	1.73	337	0.70	2.77	3.47	0.78	B	10	40	48	5
7*	26	M	100	1.80	375	0.94	2.96	3.90	1.44	B	20	36	36	7
8*	28	M	104	1.87	355	0.95	3.40	4.35	1.23	B	10	57	48	5
9*	32	M	92	1.80	260	0.95	3.74	4.68	1.57	B	10	44	48	3
10*	48	M	105	1.90	274	1.29	5.04	6.33	1.21	B	23	38	36	7
11	26	M	83	1.60	252	2.46	7.36	9.82	1.48	A	0.5	35	40 days	—
12	22	M	100	1.67	218	1.92	9.29	11.21	1.79	A	None	—	—	—
13	26	M	107	1.88	212	3.05	10.23	13.28	1.06	A	None	—	—	—
Mean	33		103	1.76	281				1.16					
SEM	2.6		3	0.04	14				0.09					
Range	(22–49)		(83–124)	(1.45–2.00)	(212–375)	(0.25–3.05)	(0.66–10.23)	(0.91–13.28)	(0.78–1.79)					
Fasting controls (N = 26)														
Mean	25	14M, 12F	101	1.83	71				1.07	15A, 11B				
SEM	7		3	0.03	2				0.06					
Range	(17–32)		(80–128)	(1.55–2.32)	(45–96)	(0.03–2.40)	(0.06–9.50)	(0.09–11.90)	(0.50–1.94)					

*Diabetic subjects for whom C-peptide and glucagon levels are available (see text).
†A, ^{14}C-acetoacetate; B, ^{14}C-β-hydroxybutyrate.
For controls, duration of fasting varied between 15 h (0 days) and 23 days (mean 3.9 days).

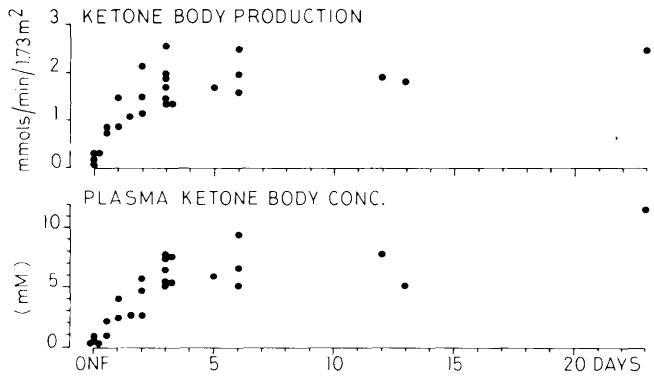

FIGURE 1. Plasma concentration and rate of production of total ketone bodies (ACAC + β-OHB) as a function of the duration of the fast in control subjects. ONF, overnight fast (15 h).

as to obtain a wide range of KB levels. Only 3 subjects fasted for more than 6 days (Figure 1).

Among the subjects composing the control group, 11 took part in exercise experiments that are already published.[3,4] Only the basal preexercise period of these studies was used here. The three subjects having fasted 12, 13, and 23 days (Figure 1) were taken from a previous study on fasting,[5] and two of them were moderately overweight (122% and 128% of ideal body weight).

Before giving their consent to participate, all the diabetic and control subjects were fully instructed as to the purpose, nature, and risks of all procedures used.

Methods. Total KB turnover was measured using a primed, continuous infusion of sodium 3-^{14}C-acetoacetate (Radiochemical Centre, Amersham, United Kingdom) or potassium D(−)-3-^{14}C-β-hydroxybutyrate (New England Nuclear, Boston, Massachusetts) (Table 1). The tracers were diluted in saline and delivered in an antecubital vein at the rate of 0.79 ± 0.04 μCi/min (mean ± SEM). The priming dose represented 20–103 times the amount infused per minute, depending on the expected degree of hyperketonemia. After the tracer had been allowed to equilibrate for at least 60 min (60–105 min), 3–4 blood samples were obtained over a period of 30–60 min. The blood was drawn from an indwelling needle located either in the brachial artery (2 diabetic subjects, 11 controls) or in an antecubital vein (11 diabetic subjects, 15 controls) of the arm opposite that used for the infusion. The injected solutions were prepared and sterilized as previously described.[6]

The methods used for determining the plasma concentration of glucose, acetoacetate (ACAC), ^{14}C-ACAC, β-hydroxybutyrate (β-OHB), ^{14}C-β-OHB, C-peptide, and glucagon (IRG) have been described before.[3] The plasma free fatty acid (FFA) levels were determined by titration after a double-extraction procedure that prevents interference of ketone bodies.[7] The recovery of ^{14}C-ACAC and ^{14}C-D(−)-βOHB added to nonradioactive plasma (obtained from the subject at the start of the study) was determined with each set of assays and was found to average 72.8 ± 1.7% for ACAC and 76.8 ± 0.9% for β-OHB. Values for ketone radioactivity in plasma were corrected accordingly.

In 6 diabetic and 15 control subjects, unlabeled and labeled ACAC and β-OHB was assayed on whole blood rather than on plasma. Therefore, the plasma/whole blood concentration ratio was determined in 15 samples with total KB levels varying between 1.40 and 9.49 mM, and it was found to average 1.24 ± 0.01 (range 1.19–1.29) for ACAC and 1.24 ± 0.01 (range 1.17–1.43) for β-OHB. Thus, a factor of 1.24 was used to convert whole blood into plasma concentrations. As expected, we could not detect any difference between whole blood and plasma ketone specific activity during ^{14}C-ketone infusions.

Calculations. In each individual experiment, the change in total KB-specific activity with time (ΔSA/Δt) was calculated by linear regression[8] using the 3–4 determinations available; it was found to be less than ±0.5%/min in all cases. On an average, ΔSA/Δt amounted to +0.15 ± 0.06%/min in diabetic subjects and to +0.06 ± 0.05%/min in the controls and the difference between the two groups was not significant (unpaired *t*-test,[8] $P > 0.05$). In the presence of this very near steady-state situation, the turnover rate of total KB (rate of appearance [Ra] = rate of disappearance) was calculated as the ratio between the amount of radioactivity infused per minute and the average specific activity of total ketones. The rate of metabolic disposal (Rd) was obtained by subtracting the urinary loss from the rate of disappearance, and the metabolic clearance rate (MCR) represents the ratio between Rd and the average total ketone concentration. All values were normalized to a body surface of 1.73 m^2. Variance is expressed as standard error of the mean (SEM). Correlation coefficients were calculated according to Snedecor.[8]

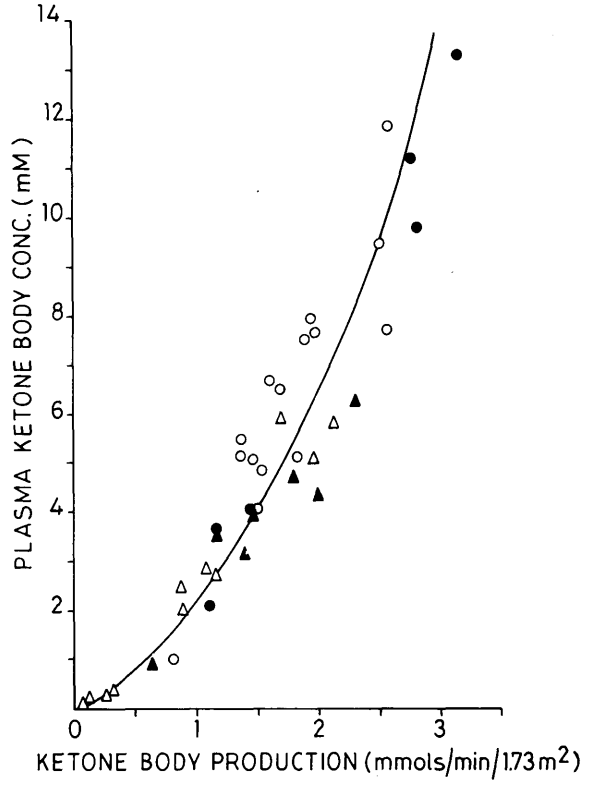

FIGURE 2. Relationship between the rate of production and the plasma concentration of total ketones in insulin-dependent diabetic subjects (closed symbols) and in normal fasted controls (open symbols). The production rate of ketones was measured using an infusion of ^{14}C-ACAC (circles) or ^{14}C-D(−)-β-OHB (triangles).

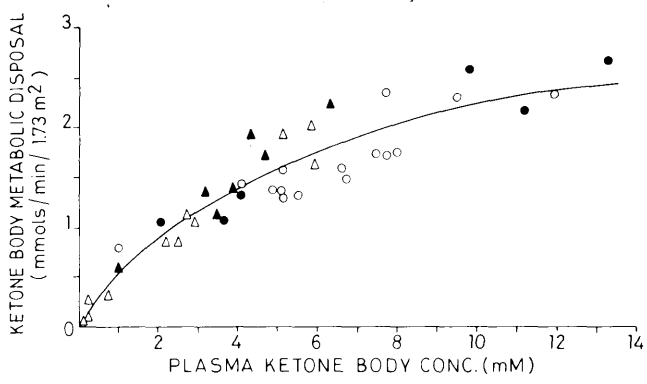

FIGURE 3. Relationship between the concentration and the metabolic disposal rate of total ketones in insulin-dependent diabetic subjects and in normal fasted controls (same symbols as in Figure 2).

RESULTS

In the diabetic group (Table 1), total KB concentration ranged between 0.91 and 13.28 mM, the β-OHB/ACAC ratio averaging 3.54 ± 0.18 in the 11 patients whose ketonemia exceeded 2 mM. FFA levels varied between 0.78 and 1.79 mM, and those of glucose between 212 and 375 mg/dl. C-peptide and IRG concentrations were determined only in the 7 diabetic subjects identified in Table 1. All were C-peptide negative except the least ketotic one (no. 1: C-peptide = 0.25 pg/ml). IRG concentrations averaged 152 ± 13 pg/ml.

In the control group, ketonemia varied between 0.09 and 11.90 mM, and 22 subjects had total KB levels exceeding 2 mM with an average β-OHB/ACAC ratio (3.72 ± 0.18) comparable to that observed in the diabetic subjects. FFA levels ranged between 0.50 and 1.94 mM. IRG concentration was measured in 6 fasting subjects with ketone levels between 2.2 and 5.9 mM and averaged 208 ± 33 pg/ml.

In all subjects with total KB levels exceeding 2 mM, the two ketone bodies were in isotopic equilibrium during the infusion of ^{14}C-β-OHB, the β-OHB/ACAC SA ratio averaging 1.02 ± 0.03 in the diabetic subjects (N = 6) and 1.05 ± 0.04 in the controls (N = 7). Equilibrium was not observed during ^{14}C-AÇAC infusions, but the SA ratios were similar in diabetic subjects (0.48 ± 0.03, N = 6) and normals (0.47 ± 0.02, N = 14).

Figure 1 represents the concentrations and the rate of production of total KB in relation to the duration of the fast in the control group. Both parameters reached a plateau after 3 days. One exception was the slightly obese subject fasted for 23 days, in whom KB level (but not Ra) exceeded that of all the other subjects. It should be pointed out that, ignoring this case, the full range of concentrations and turnover rates of ketones studied in this group was obtained by a fast of <1 wk.

Figure 2 depicts the curvilinear relationship existing between Ra and total KB concentration in normal and diabetic subjects. The two groups seemed to behave similarly and there was no obvious influence of the type of tracer used on the observed results. In the most ketotic starved subject, plasma total KB concentration attained 11.9 mM at a rate of production of 2.5 mmol/min/1.73 m^2, corresponding values being 13.3 mM and 3.1 mmol/min/1.73 m^2 in the most ketotic diabetic patient. About one-half this amount is produced by starved or diabetic subjects at a ketone concentration of 4 mM.

A positive correlation between urinary excretion and plasma concentration of total ketones was observed in diabetic subjects ($r = 0.90$, $P < 0.001$) and in controls ($r = 0.69$, $P < 0.001$). In both groups, urinary elimination represented <6% of Ra at plasma levels below 4 mM and <12% at ketone concentrations below 10 mM. In the two most ketotic diabetic subjects (11.2 and 13.3 mM), urinary loss amounted, respectively, to 20% and 16% of total ketone turnover. Interestingly, only one-third this amount was found in the urine of the 23-day fasted, nondiabetic subject who had similar ketone levels (11.9 mM).

Figure 3 represents the rate of metabolic disposal of ketones (Rd) in relation to total KB concentration. Again, no significant difference was observed between control and diabetic subjects. This saturation-type curve shows that a maximal metabolic disposal rate of about 2.3 mmol/min/1.73 m^2 is attained in both groups for KB concentrations exceeding 9 mM, the half-maximal Rd being observed at a concentration of about 3 mM.

The MCR, which is a useful index of the ability of tissues to remove the substrate from plasma,[9] decreases when ketone concentration rises, as shown in Figure 4. At low ketone levels, such as those observed in overnight-fasted subjects (0.1–0.2 mM), metabolic clearance rates as high as 1200–1300 ml/min/1.73 m^2 were observed. Any increase in KB levels in the low range of values resulted in a dramatic drop in MCR. For KB levels exceeding 2 mM, the MCR continued to fall with rising ketonemia, but at a much lower rate; for instance, a rise in total KB levels from 2 to 11 mM resulted in only a modest fall in MCR from 400 to 250 ml/min/1.73 m^2, no difference being observed between diabetic and control subjects.

The relationship between plasma FFA levels and Ra is shown in Figure 5. These parameters were significantly correlated both in the diabetic subjects ($r = 0.62$, $P < 0.05$) and in the fasted controls ($r = 0.64$, $P < 0.001$). No difference could be detected between the two groups.

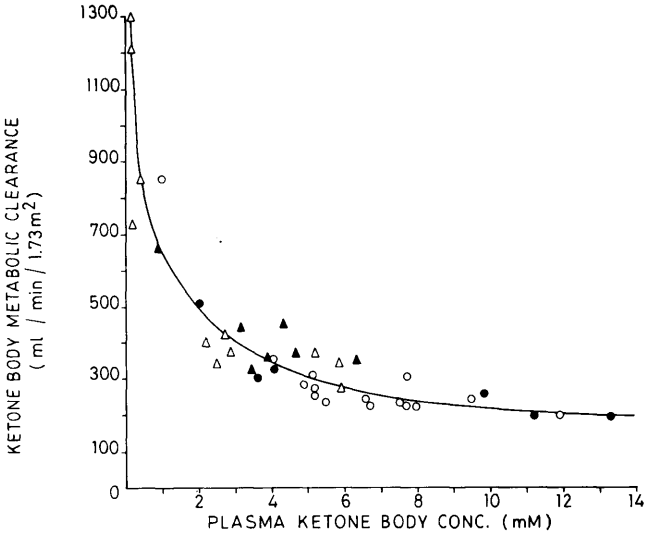

FIGURE 4. Metabolic clearance rate of total ketones as a function of their plasma concentration in insulin-dependent diabetic subjects and in normal fasted controls (same symbols as in Figure 2).

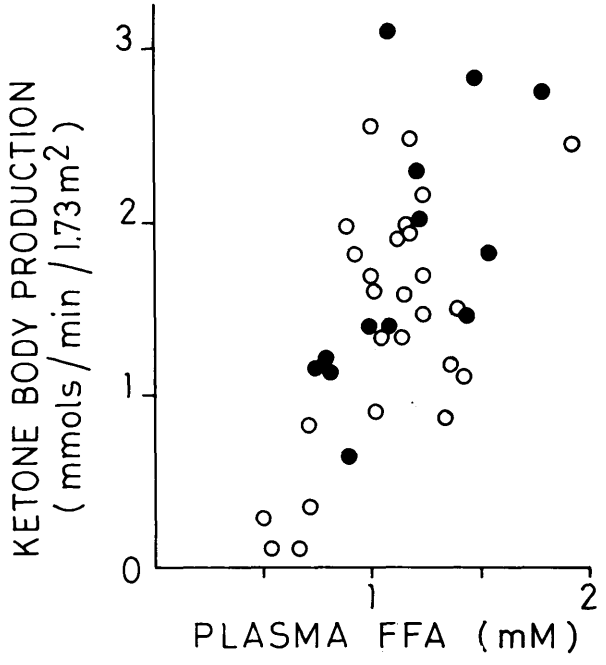

FIGURE 5. Correlation between plasma FFA levels and the rate of production of total ketone bodies in insulin-dependent diabetic subjects (closed circles) and in normal fasted controls (open circles).

DISCUSSION

This work compares the kinetics of KB in 13 insulin-deprived, type I diabetic subjects and 26 fasting, nonobese subjects at KB levels varying between 1 and 12 mM. None of the diabetic patients was in ketoacidosis.

The use of the combined specific activity of ACAC and β-OHB for measuring total KB turnover during ^{14}C-ACAC or ^{14}C-β-OHB infusions poses methodologic problems that have been discussed in several previous papers.[3-5,10] Experimental validation of this technique has been provided in normal and diabetic dogs[11,12] and in man.[5,13] The present data further confirm that there is no systematic discordance between turnover rates obtained with the two tracers (Figures 2–4), despite important differences in the degree of isotopic equilibration reached. The use of venous instead of arterial blood for ketone determinations in some experiments should not influence our results appreciably. Indeed, it is known[14] that the specific activity of ketones and, therefore, their estimated turnover rates, are not influenced by the site of sampling. On the other hand, the difference in unlabeled ketone concentration between artery and vein (which would influence the calculations of MCR) should not exceed a few percent in ketotic subjects.[15,16]

To define the perturbations that can lead to uncontrolled hyperketonemia in type I diabetic subjects, it seems appropriate to first analyze the kinetics of KB in our fasting control subjects.

The plasma ketone level is related to Ra in an exponential manner over the whole range of concentrations observed (Figure 2). This exponential pattern results from the fact that the removal capacities of tissues are progressively saturated as concentration rises (Figure 3). Thus, the hyperketonemia of fasting is primarily caused by an increased production of ketones, but the phenomenon is amplified by a progressive limitation in the ability of tissues to extract ketones from blood.

Fasting ketosis has the remarkable property of being self-limited, the time needed to attain the maximal hyperketonemia depending on the experimental conditions. Once the steady state is attained, plasma total KB concentration amounts to about 10–12 mM and uptake by tissues has reached its maximal value (maximal Rd), which approximates 2.3 mmol/min/1.73 m^2 (Figure 3). At this degree of ketosis, any small increase in Ra would result in a marked increase in concentration (Figure 2). The conditions are, thus, fulfilled, under which the inhibitory action of the hyperketonemia on the ketogenesis of fasting[6] has its greatest efficacy. The overall effect of this physiologic regulatory system during starvation is to maintain Ra at values (about 2.5 mmol/min/1.73 m^2) only slightly above maximal Rd, the difference being accounted for by urinary excretion.

The dependence of the ketone removal capacities on the concentration is best shown by Figure 4, which depicts the curvilinear decay of MCR in relation to total KB level. Up to a concentration of 1–2 mM, the curve has a very steep slope, a much slower decrement of MCR being observed above 5 mM. This pattern is compatible with the concept that some tissues have a high affinity for ketones at low concentration but rapidly become saturated as concentration rises, while other tissues have a capacity to clear ketones that is more limited at low concentration but also much less depressed by an increase in ketonemia. Muscle may belong to the first type of tissue, since it has been shown in man that the extraction ratio of ketones by muscle ([A − V]/A) decreases from 50% at 0.1 mM to 1–2% at 6–7 mM.[15-18] On the contrary, the extraction ratio of KB by some other tissues, such as the brain, approximates 5–10% whatever the ketone concentration.[19,20] This differential behavior of two tissues playing a major role in ketone consumption might be the basis of the redistribution of this fuel from muscle to brain with increasing ketonemia, a phenomenon that has been shown to occur during progressive fasting.[15,20]

The data of Owen et al.[21,22] on the kinetics of KB during fasting differ from ours in several respects. These authors studied mainly very obese, hospitalized patients fasted for short (2–3 days) or long (several weeks) periods. They observed that beyond a KB concentration of 3–4 mM (attained after about 3 days), ketogenesis became steady, while KB levels continued to rise for several weeks, so that Ra was no longer related to ketonemia. They concluded that the rise in KB levels occurring after more than 3 days of fasting was entirely related to a reduction in peripheral removal. The much more rapid development of maximal fasting hyperketonemia observed in our studies (Figure 1) is probably related to the absence of obesity and even more so to the unrestricted physical activity of our subjects during the fast. In our experiments, a significant correlation was found between KB concentration and Ra even if only ketone levels above 4 mM were taken into account ($r = 0.75$, $P < 0.001$). Therefore, the increase in KB levels above 4 mM can not be attributed entirely to a removal defect. There is no doubt, however, that a progressive impairment of removal participates to the hyperketonemia of fasting, but the MCR data (Figure 3) show that this phenomenon contributes to the rise in ketonemia even at low levels of ketones, i.e., from the very

beginning of the fast. Furthermore, the decrease in MCR associated with the rise in KB levels is caused by hyperketonemia itself rather than by fasting, since we observed the same phenomenon in a model of artificial ketosis induced in overnight-fasted subjects by the infusion of exogenous ketones.[5]

In our group of diabetic subjects, the range of FFA levels, the relationship between FFA and Ra, and the curves relating Ra, Rd, and MCR to KB concentration were very similar to those observed in the fasting group (Figures 2–5). Thus, there is no evidence for a ketone removal defect in diabetic subjects when compared with fasting subjects at the same ketone concentration, and the maximal rate of disposal approximates 2.3–2.5 mmol/min/1.73 m^2 for the two groups. Miles et al.[2] and Keller et al.[23] also noticed a progressive impairment in the metabolic clearance of ketones in the presence of rising KB concentration in acutely insulin-deprived type I diabetic subjects. They suggested that this phenomenon was related to the gradual insulin deficiency that occurs in these patients. It is probable that, in addition to this mechanism,[24] hyperketonemia itself plays an even more important role in this process, as already mentioned.

Since the maximal Rd in diabetic subjects is similar to that observed in starved controls, it seems obvious that the much higher KB levels that can be observed in decompensated diabetic subjects must result from higher rates of ketogenesis, a notion that has been recently emphasized by Miles et al.[25] The fact that markedly ketotic diabetic subjects have much higher levels of FFA and glucagon than those observed here[26] also supports the idea that they produce unphysiologically high amounts of ketones.

Owen et al.[1] measured splanchnic production of KB in a diabetic population that was different from ours, as their patients were acidotic and dehydrated, whereas the patients in the current study were not. These authors concluded that the major determinant of diabetic hyperketonemia was an impaired removal of ketone bodies. This conclusion is based in part on the observation that their ketotic diabetic subjects had rates of ketogenesis comparable to those observed in 3-day-fasted subjects whose concentrations were five times lower.[27] However, other data of Owen's group[28] show that, after prolonged fasting, the splanchnic production and the concentration of ketones are comparable to those of several of his diabetic subjects,[1] clearly indicating that there is no systematic difference in the capacity to utilize ketones between fasted subjects and diabetic patients if they are compared at the same elevated ketone levels, an observation that is in keeping with our data.

With regard to absolute levels of ketogenesis, it should be noted that our KB production rates are 2–3 times greater than those observed by other investigators,[22,28] but only at KB levels exceeding 3 mM. As discussed before,[5,6] the reason for this difference remains unknown. Obviously, the oxidation of such large amounts of KB exceeds total metabolic requirements during starvation. Since fuels other than ketones are also oxidized, one is led to hypothesize that a significant proportion of KB taken up by the tissues escapes from oxidation and is channeled into synthetic pathways. In vitro data have shown that, under certain conditions, KB can serve as precursors for lipid synthesis in various organs such as brain, adipose tissue, and liver,[29,30] but the quantitative importance of these pathways in adult fasted humans is totally unknown.

There is also disagreement in the literature with regard to absolute levels of ketogenesis in diabetic ketosis. For instance, Keller et al. (using ^{14}C-ACAC)[23] and Miles et al. (using ^{14}C-β-OHB)[2] reported almost identical ketone production rates (around 1.2 mmol/min) in type I diabetic patients several hours after interruption of an insulin infusion, but there was a more than threefold difference in the concentration of ketones, which averaged 2.2 mM in the first study and more than 7 mM in the second. Our values are intermediate between these two groups of data.

Whatever its cause may be, the great variability in KB turnover rates observed between different studies should not affect our comparison between fasting and diabetic ketosis, since both groups were studied with the same technique.

In conclusion, the patterns of KB kinetics are similar in fasting and diabetes for ketone concentrations ranging between 1 and 10 mM. In both situations, hyperketonemia is caused primarily by increased production having effects on ketonemia that are progressively amplified by the fall in the metabolic clearance rate associated with an elevation in KB levels.

Both groups have a similar maximal metabolic disposal rate approximating 2.3 mmol/min/1.73 m^2. The homeostatic mechanism(s) that maintain(s) Ra very close to maximal Rd in fasting ketosis must be altered in severely insulin-deprived diabetic subjects in whom ketonemia can largely exceed that observed during starvation. Our data favor the concept that excessive production of KB represents the main factor leading to uncontrolled hyperketonemia.

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REFERENCES

[1] Owen, O. E., Block, B. S. B., Patel, M., Boden, G., McDonough, M., Kreulen, T., Shuman, C. R., and Reichard, G. A., Jr.: Human splanchnic metabolism during diabetic ketoacidosis. Metabolism 1977; 26:381–98.

[2] Miles, J. M., Rizza, R. A., Haymond, M. W., and Gerich, J. E.: Effects of acute insulin deficiency on glucose and ketone body turnover in man. Evidence for the primacy of overproduction of glucose and ketone bodies in the genesis of diabetic ketoacidosis. Diabetes 1980; 29:926–30.

[3] Féry, F., and Balasse, E. O.: Ketone body turnover during and after exercise in overnight-fasted and starved humans. Am. J. Physiol. 1983; 245:E318–25.

[4] Balasse, E. O., Féry, F., and Neef, M. A.: Changes induced by exercise in rates of turnover and oxidation of ketone bodies in fasting man. J. Appl Physiol. 1978; 44:5–11.

[5] Balasse, E. O.: Kinetics of ketone body metabolism in fasting humans. Metabolism 1979; 28:41–50.

[6] Balasse, E. O., and Neef, M. A.: Inhibition of ketogenesis by ketone bodies in fasting humans. Metabolism 1975; 24:999–1007.

[7] Trout, D. L., Estes, E. H., and Friedberg, S. J.: Titration of free fatty acids of plasma: a study of current methods and a new modification. J. Lipid Res. 1960; 1:199–202.

[8] Snedecor, G. W.: Statistical Methods, 5th edit. Ames, Iowa, Iowa State University Press, 1956.

[9] Issekutz, B., Jr., Issekutz, T. B., and Elahi, D.: Glucose kinetics during oral glucose tolerance test in normal, methylprednisolone-treated and alloxan-diabetic dogs. Diabetes 1974; 23:645–50.

[10] Balasse, E. O., and Delcroix, C.: Isotopic study of ketone body ki-

netics: controversy on methodological aspects. Metabolism 1980; 29:395–96.

[11] Keller, U., Cherrington, A. D., and Liljenquist, J. E.: Ketone body turnover and net hepatic ketone production in fasted and diabetic dogs. Am. J. Physiol. 1978; 235:E238–47.

[12] Miles, J. M., Haymond, M. W., Rizza, R. A., and Gerich, J. E.: Determination of ^{14}C-radioactivity in ketone bodies: a new simplified method and its validation. J. Lipid Res. 1980; 21:646–50.

[13] Keller, U., Sonnenberg, G., and Stauffacher, W.: Validation of a tracer technique to determine nonsteady-state ketone body turnover rates in man. Am. J. Physiol. 1981; 240:E253–62.

[14] Sonnenberg, G. E., and Keller, U.: Sampling of arterialized heated-hand venous blood as a noninvase technique for the study of ketone-body kinetics in man. Metabolism 1982; 31:1–5.

[15] Owen, O. E., and Reichard, G. A., Jr.: Human forearm metabolism during progressive starvation. J. Clin. Invest. 1971; 50:1536–45.

[16] Hagenfeldt, L., and Wahren, J.: Human forearm muscle metabolism during exercise. IV. Substrate utilization in prolonged fasting. Scand. J. Clin. Lab. Invest. 1971; 27:299–306.

[17] Rennie, M. J., Park, D. M., and Sulaiman, W. R.: Uptake and release of hormones and metabolites by tissues in exercising leg in man. Am. J. Physiol. 1976; 231:967–73.

[18] Hagenfeldt, L., and Wahren, J.: Human forearm muscle metabolism during exercise. III. Uptake, release and oxidation of β-hydroxybutyrate and observations on the β-hydroxybutyrate/acetoacetate ratio. Scand. J. Clin. Lab. Invest. 1969; 21:1–8.

[19] Dietze, G., Wicklmayr, M., and Mehnert, H.: On the key role of ketogenesis for the regulation of glucose homeostasis during fasting: intrahepatic control, ketone levels and peripheral pyruvate oxidation. *In* Biochemical and Clinical Aspects of Ketone Body Metabolism. Söling, H. D., and Seufert, C. D., Eds. Stuttgart, G. Thieme, 1978:213–25.

[20] Owen, O. E., Morgan, A. P., Kemp, H. G., Sullivan, J. H., Herrera, M. G., and Cahill, G. J., Jr.: Brain metabolism during fasting. J. Clin. Invest. 1967; 46:1589–95.

[21] Owen, O. E., Caprio, S., Reichard, G. A., Jr., Mozzoli, M. A., Boden, G., and Owen, R. S.: Ketosis of starvation: a revisit and new perspectives. Clin. Endocrinol. Metab. 1983; 12:359–79.

[22] Reichard, G. A., Jr., Owen, O. E., Haff, A. C., Paul, P., and Bortz, W. M.: Ketone-body production and oxidation in fasting obese humans. J. Clin. Invest. 1974; 53:508–15.

[23] Keller, U., Schnell, H., Sonnenberg, G. E., Gerber, P. P. G., and Stauffacher, W.: Role of glucagon in enhancing ketone body production in ketotic diabetic man. Diabetes 1983; 32:387–91.

[24] Balasse, E. O., and Havel, R. J.: Evidence for an effect of insulin on the peripheral utilization of ketone bodies in dogs. J. Clin. Invest. 1971; 50:801–13.

[25] Miles, J. M., and Gerich, J. E.: Glucose and ketone body kinetics in diabetic ketoacidosis. Clin. Endocrinol. Metab. 1983; 12:303–19.

[26] Foster, D. W., and McGarry, J. D.: The metabolic derangements and treatment of diabetic ketoacidosis. N. Engl. J. Med. 1983; 309:150–59.

[27] Garber, A. J., Menzel, P. H., Boden, G., and Owen, O. E.: Hepatic ketogenesis and gluconeogenesis in humans. J. Clin. Invest. 1974; 54:981–89.

[28] Owen, O. E., Felig, P., Morgan, P., Wahren, J., and Cahill, G. F., Jr.: Liver and kidney metabolism during prolonged starvation. J. Clin. Invest. 1969; 48:574–83.

[29] Robinson, A. M., and Williamson, D. H.: Physiological role of ketone bodies as substrates and signals in mammalian tissues. Physiol. Rev. 1980; 60:143–87.

[30] Endemann, G., Goetz, P. G., Edmond, J., and Brunengraber, H.: Lipogenesis from ketone bodies in the isolated perfused rat liver. J. Biol. Chem. 1982; 257:3434–40.