Inhibition of Ketogenesis by Ketone Bodies in Fasting Humans

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Although there exists some indirect evidence that circulating ketone bodies might inhibit their own production rate, the direct demonstration of this homeostatic feed-back phenomenon is still lacking. The present work aims at demonstrating the operation of this control mechanism in human fasting ketosis.

Six obese subjects, who fasted 2-23 days, were given a primed constant i.v. infusion of 3-14C-acetoacetate for 4 hr. After a control period of 2 hr, unlabeled sodium acetoacetate was administered as a primed constant i.v. infusion at the rate of 0.688–1.960 mmol/min until the end of the study. During both periods, the rates of inflow of ketones were estimated from the specific activity of total ketones measured under near isotopic steady state conditions.

During the control period, total ketone concentration amounted to 3.98–9.65 μmol/ml and production rates of total ketones ranged between 1.450 and 2.053 mmol/min. The levels of free fatty acids, glycerol, glucose, and insulin averaged respectively 1.30 μmol/ml, 0.11 μmol/ml, 74 mg/100 ml, and 5.2 μU/ml.

The administration of exogenous ketones during the second phase of the study induced a 47%–92% increase in total ketone levels. During this period, the endogenous production of ketones (calculated as the difference between total inflow rate and acetoacetate infusion rate) amounted only to 67%–90% of control values. Among other factors, this inhibition of ketogenesis was probably partially related to the direct antilipolytic effect of infused ketones. Indeed, there was a concomitant fall in FFA and in glycerol levels averaging respectively 13.5% and 17.3%, without significant changes in peripheral insulin concentrations.

Our results demonstrate that during fasting, circulating ketone bodies exert an inhibitory influence on the rate of ketogenesis. This mechanism might play an important role in preventing the development of uncontrolled hyperketonemia during starvation.
Since approximately 10 years, it has been repeatedly observed that the infusion of ketone bodies reduce plasma FFA levels in various animal species including man. Since plasma FFA are the primary ketogenic substrate, it has been speculated that the ketone-induced inhibition of adipose tissue lipolysis might restrain ketogenesis. If this were true, this feed-back mechanism should play an important homeostatic role during starvation by preventing the development of uncontrolled hyperketonemia. However, direct evidence for the operation of this feed-back cycle has never been provided. The present experiments demonstrate that in fasting man, the rate of ketogenesis, as measured by an isotopic technique, is indeed inhibited by an exogenous supply of ketones.

MATERIALS AND METHODS

The study was performed on 6 obese nondiabetic female subjects aged 17-46 yr, hospitalized for therapeutic starvation. Their body weight varied between 115% and 164% of ideal body weight at the time of the study. During the fast, the subjects had a daily intake of one multivitamin capsule (Maxivit, Roerig, Belgium) and were allowed to drink calorie-free beverages (water, black coffee, or tea) ad libitum until 12 hr before the test. Only water was allowed thereafter.

The experiments were performed after various durations of fast ranging from 2 to 23 days. An indwelling Cournand needle was placed in a brachial artery for blood sampling and two venous cannulae were inserted in the opposite arm for infusions.

The studies were conducted according to the following protocol (Fig. 1). After a first arterial blood specimen had been obtained, sodium 3-14C-acetoacetate was infused at a constant rate (0.41-0.57 μCi/min) for 4 hr. A priming dose of label corresponding to the amount infused in 80 min was given at the start of the infusion. After a basal period of 2 hr, a primed constant infusion of unlabeled sodium acetoacetate (AcAc) was superimposed to the isotope infusion. The rate of delivery ranged between 0.688 and 1.960 mmol/min, the priming dose corresponding to 80 times the amount infused per min.

Four 25 ml blood samples were collected at 15 min intervals during the last 45 min of each period and were placed in heparinized tubes and immediately chilled. Samples of 5 ml blood were mixed with perchloric acid and neutralized and the resulting protein-free filtrate was kept on ice until further use. The rest of the blood was centrifuged and the plasma was analyzed for content of FFA, glucose, and immuno-reactive insulin (IRI). Glycerol, AcAc, and β-hydroxybutyrate (βOHB) concentrations were estimated in the perchloric filtrate using enzymatic methods.

14C in ketone bodies was measured by the method of Mayes and Felts. This method permits the determination of 14C activity separately in AcAc and βOHB; 14C-βOHB is converted enzymatically to 14C-AcAc; 14C-AcAc is decarboxylated to acetone and CO2 which are trapped separately in a double-well flask and assayed for 14C in a liquid scintillation spectrometer. This original method was slightly modified along two lines: (1) since the AcAc used in these experiments was labeled on the carbonyl carbon, only the labeled acetone produced was measured; (2) the procedure was adapted to handle larger volumes of perchloric filtrate, so that ultimately the radioactivity measured corresponded to that contained in 0.5 ml of blood instead of 0.05-0.06 ml of blood for the original method. The recovery of 14C-AcAc and DL-14C-βOHB added to nonradioactive blood (obtained from the patient at the start of the study) was determined with each set of assays and found to be 63%-89% (mean 77%) for AcAc and 76%-89% (mean 81%) for βOHB. Recoveries were consistent within an experiment. For βOHB, recovery was calculated taking into account the fact that the assay is specific for the D(-) isomer so that only half of the added 14C was expected to be recovered. Values for ketone radioactivity in blood samples were corrected accordingly. Any 14C-acetone which could be present in the perchloric filtrate before determination of 3-14C-AcAc and 3-14C-βOHB, was removed by prior ventilation of the filtrate with a stream of nitrogen for at least 1 hr. The determinations of unlabeled and labeled AcAc were done on the day of each experiment owing to the instability of the compound.
Fig. 1. Average experimental results in six subjects.

Blood pH was measured at the end of the control period and at the end of the AcAc infusion period, in four of the six patients studied. All analyses were performed in duplicate.

Preparation of materials for injection. The unlabeled and the labeled sodium AcAc were prepared, respectively, from unlabeled ethyl AcAc (Merck, Darmstadt, Germany) and chromatographically pure ethyl $3^{14}$C-AcAc (The Radiochemical Center, Amersham, England, specific
activity 12.7 mCi/mmol), according to previously described techniques. The radiochemical purity of the prepared $^{14}$C-AcAc solutions was determined according to Mayes and Felts and found to vary between 93% and 97%. The radioactivity was mixed with a small volume (2-3 ml) of 0.5 M AcAc in order to minimize its degradation. After preparation, the solutions were sterilized through a Millipore filter (0.22 μ) and kept frozen at −20°C until use. Immediately before use, the unlabeled AcAc was diluted with distilled water so as to obtain a concentration varying between 222 and 631 μmol/ml and the labeled AcAc was diluted with saline. All infused solutions had a pH of 7–7.5 and were kept chilled with ice during the course of the infusions.

Calculations. During the last 45 min of both phases of the experiments, the specific activity (S.A.) of total ketone bodies was calculated for each time point as follows: S.A. of total ketones (dpm/μmol) = $^{14}$C-AcAc (dpm/ml) + $^{14}$C-βOHB (dpm/ml)/[AcAc (μmol/ml) + βOHB (μmol/ml)].

Although on an average, the S.A. of total ketones had almost attained the steady state during both periods (Fig. 1), a significant rise was nevertheless observed in several experiments. Therefore, for both periods of each experiment, the production rate of total ketones was calculated according to the equation proposed by Steele which allows for correction for the change in S.A. with time: $R = \frac{I - (C \times V \times dS.A./dt)}{S.A.}$ (II) where $R$ is the rate of appearance (or inflow) of ketones (μmol/min), $I$ is the infusion rate of the tracer (dpm/min), $C$ is the average concentration of total ketones over the studied period (μmol/ml), $V$ is the “operational” volume of distribution of ketones (ml) which was shown to approximate 20% of body weight in fasted subjects, $dS.A./dt$ represents the increment in S.A. as a function of time and was calculated by linear regression using the four values obtained during the last 45 min of each period, and S.A. is the average S.A. of total ketones over the studied period (dpm/μmol).

During the basal period, only the isotope was infused and the rate of appearance of total ketones thus represents the rate of ketogenesis.

During the second phase, the rate of appearance of total ketones represents the sum of the exogenous AcAc inflow and of the endogenous ketone production. The latter was thus obtained by subtracting the exogenous AcAc inflow from the rate of appearance of total ketones.

The rationale for using the S.A. of total ketones instead of that of AcAc in calculating the inflow rate of ketones under our experimental conditions will be outlined under the discussion section.

Statistical significance of the observed changes was tested by paired comparison (Student’s t test) of the mean data obtained in each subject during the two experimental periods.

RESULTS

During the basal period, the AcAc and βOHB concentrations were steady in all subjects (Fig. 1) and total ketone concentrations ranged between 3.98 and 9.65 μmol/ml (Table 1). βOHB levels largely exceeded those of AcAc, the βOHB/AcAc ratio varying between 3.23 and 4.05. The S.A. of ketone bodies almost plateaued during the last 45 min of the basal period but the S.A. of βOHB did not exceed 41%-52% of that of AcAc. Ketogenesis calculated according to equation II varied between 1.450 and 2.053 mmol/min and seemed roughly independent of the duration of the fast, at least for the studied fasting periods.

The administration of unlabeled AcAc (at rates equivalent to 43%-98% of basal ketone production) induced a marked rise in both the AcAc and βOHB concentrations which plateaued during the last 45 min of the infusion (Fig. 1). Total ketone concentrations amounted to 7.41-16.44 μmol/ml during this period (Table 1). The βOHB/AcAc ratio was systematically decreased by comparison with the basal period, varying between 1.47 and 2.44. The rise in ketone concentration was associated with a fall in ketone S.A. indicating that total ketone inflow was increased. The difference between total inflow rate and ex-
Table 1. Effect of an Infusion of Sodium Acetoacetate on Blood Metabolite Concentration and Ketone Bodies Production Rates in Fasted Obese Subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Duration of Fast</th>
<th>Percent of Ideal Body Weight</th>
<th>Days</th>
<th>AcAc Infused</th>
<th>AcAc</th>
<th>βOHB</th>
<th>Percent Inhibition from Control</th>
<th>FFA</th>
<th>Glycerol</th>
<th>Glucose</th>
<th>IRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRU.</td>
<td>2</td>
<td>126</td>
<td>0</td>
<td>0.94</td>
<td>3.04</td>
<td>3.98</td>
<td>1.588</td>
<td>1.16</td>
<td>0.108</td>
<td>68</td>
<td>3.0</td>
</tr>
<tr>
<td>DEF.</td>
<td>5</td>
<td>135</td>
<td>0</td>
<td>1.31</td>
<td>5.99</td>
<td>7.30</td>
<td>1.836</td>
<td>1.36</td>
<td>0.074</td>
<td>61</td>
<td>1.8</td>
</tr>
<tr>
<td>LEN.</td>
<td>7</td>
<td>115</td>
<td>0</td>
<td>1.27</td>
<td>4.17</td>
<td>5.43</td>
<td>1.450</td>
<td>1.01</td>
<td>0.070</td>
<td>67</td>
<td>3.0</td>
</tr>
<tr>
<td>DUF.</td>
<td>14</td>
<td>164</td>
<td>0</td>
<td>1.57</td>
<td>5.58</td>
<td>7.15</td>
<td>1.993</td>
<td>1.35</td>
<td>0.140</td>
<td>78</td>
<td>13.0</td>
</tr>
<tr>
<td>DEL.</td>
<td>18</td>
<td>135</td>
<td>0</td>
<td>1.53</td>
<td>5.41</td>
<td>6.94</td>
<td>1.614</td>
<td>0.95</td>
<td>0.099</td>
<td>81</td>
<td>5.5</td>
</tr>
<tr>
<td>KAM.</td>
<td>23</td>
<td>128</td>
<td>0</td>
<td>1.91</td>
<td>7.74</td>
<td>9.65</td>
<td>2.053</td>
<td>1.94</td>
<td>0.153</td>
<td>87</td>
<td>4.8</td>
</tr>
</tbody>
</table>

*For each subject: upper line: mean of four values obtained during the last 45 min of the control period, lower line: mean of four values obtained during the last 45 min of the AcAc infusion period.
ogenous AcAc infusion rate provides a measurement of the residual rate of ketogenesis which was 10%–33% lower than that observed during the control period ($p < 0.005$). This inhibition of ketogenesis by ketone bodies was observed whatever the duration of the fast.

The infusion of AcAc inhibited adipose tissue lipolysis as evidenced by a significant fall in the average concentration of FFA ($13.5\% \pm 3.6\%; p < 0.02$) and of glycerol ($17.3 \pm 6.0\%; p < 0.05$). It should be noticed that, on an average, FFA (Fig. 1) and glycerol levels tended to return towards basal value at the end of the infusion period. In fact, this trend was apparent in only three of the six subjects tested, whereas the other subjects maintained their lowered FFA levels until the end of the ketone infusion. The degree of inhibition of ketogenesis was not significantly related to the fall in FFA levels ($p > 0.1$). A lowering of blood glucose was noticed ($6\% \pm 2\%; p < 0.05$) but there was no significant change in arterial IRI levels ($p > 0.05$). In four of the six subjects studied, the infusion of sodium AcAc induced only minimal changes in arterial pH, its mean value rising from 7.36 to 7.40.

**DISCUSSION**

The estimation of ketone bodies production rate using a constant infusion of $^{14}$C-AcAc meets with difficulties owing to the absence of isotopic equilibration between blood AcAc and $\beta$OHB. This situation has been observed by others in rats and man. The liver is believed to be the main site of interconversion between ketones and McGarry et al. and Bates provided strong evidence that the disequilibrium found in the isotopic steady state is due to a limited capacity of the liver to carry out the equilibration of infused isotope between AcAc and $\beta$OHB. Under these conditions, the S.A. of total ketones is assumed to represent the S.A. that would have been attained for both ketones if hepatic equilibration had not been limiting. The best estimate of total ketone body turnover would appear, therefore, to be derived from total ketone body S.A. and this technique of calculation has been employed by McGarry et al. in rats and by Reichard et al. in humans.

During the basal period, the plasma (blood) concentrations of FFA, glycerol, and glucose were in the accepted normal range for subjects starved 2–23 days but ketone levels tended to be slightly higher than those observed by others. The rates of production of ketones (1.450–2.053 mmol/min) were significantly higher than those published by Reichard et al. (0.674–1.553 mmol/min) although these authors used an isotopic technique similar to ours. The reasons for these differences are not obvious. Most of the obese subjects studied by this latter group had a much greater excess body weight and had higher IRI levels than those studied here and this might account, at least partly, for the observed differences. It should be mentioned that in another group of eight obese subjects fasted 3–23 days studied in our laboratory, rates of ketogenesis measured using either constant infusions or single injections of $^{14}$C-AcAc, were of the same order of magnitude (1.670–2.946 mmol/min) as those observed here. Whatever mechanism may be responsible for the higher ketone turnover rates found in our subjects, it should not interfere with the main significance of this work which is based on the comparison of rates of production obtained in the same subject under two different experimental conditions.
The administration of AcAc induced a rise in ketonemia and a significant and sustained drop in ketone production rate (Table 1). This effect was observed whatever the duration of the fast. The decrease in ketone production rate is likely to result, at least partly, from the observed fall in the levels of FFA which are known to be the major ketogenic substrate. Although an insulino-tropic effect of ketone bodies can undoubtedly be observed in man under appropriate conditions,\textsuperscript{10,11,26,27} the fall in FFA found in our experiments cannot be explained on this basis, peripheral IRI levels remaining steady or even decreasing in some patients during the AcAc infusion. Under these conditions, the drop in FFA was probably related to the direct antilipolytic properties of ketone bodies first demonstrated in vitro by Björntorp\textsuperscript{28} and confirmed by many others.\textsuperscript{29-31} A fall in FFA levels independent of insulin release following ketone infusions in man has been previously described.\textsuperscript{7,8,12} The participation of a ketone-induced inhibition of glucagon secretion\textsuperscript{32,33} in the observed antilipolytic effect of ketones cannot be ruled out in these experiments.

The antiketogenic effect of AcAc infusions can probably not be explained solely on the basis of their antilipolytic action for the two following reasons: (1) in three of the six subjects tested, the antilipolytic effect was transient although ketogenesis remained depressed until the end of the ketone infusion; (2) there was no correlation between the fall in FFA levels and the corresponding degree of inhibition of ketone production; for instance, the marked decrease in ketogenesis observed in subjects DEL and KAM (Table 1) was not associated with a significant drop in FFA levels. Additional mechanism(s) should therefore be considered in order to explain the observed reduction in ketogenesis; one possibility is that, despite the absence of IRI changes in peripheral blood, the ketone infusion could have stimulated insulin release in the portal vein\textsuperscript{27} thereby inhibiting hepatic ketogenesis independently of changes in the amounts of FFA delivered to the liver.\textsuperscript{34}

The observed hypoglycemic effect of ketones is confirmatory of many other studies.\textsuperscript{7-9,12,27} The data of the literature indicate that this effect may be accompanied, but need not depend upon the release of insulin.\textsuperscript{7,8,12}

The influence of exogenous AcAc on endogenous ketone production has been studied before by Bergman et al.\textsuperscript{35} in sheep and by Bates\textsuperscript{23} in rats. The first group of authors found comparable rates of endogenous AcAc production in normal and AcAc infused sheep. Their experimental design has the disadvantage of comparing two different groups of animals whereas our subjects served as their own control in a single experiment. Bates noted that infused AcAc inhibits ketogenesis but she thought that the calculated reduction in endogenous appearance of AcAc could result from a methodological artefact.\textsuperscript{23}

In conclusion, these data represent the first direct demonstration that circulating ketones exert a negative feedback on the rate of ketogenesis in fasting man. This effect is probably partially mediated through the direct antilipolytic action of ketones on adipose tissue, but our data suggest that it is not the only involved factor. The physiological implications and teleological significance of such a mechanism in maintaining ketone homeostasis during starvation are obvious and have already been emphasized by several authors including us.\textsuperscript{1,6-9,12,29} The demonstration that infused ketone bodies reduce FFA levels in pancreatectomized dogs\textsuperscript{2} and in insulin-dependent diabetic humans\textsuperscript{12} suggest
that diabetic ketosis is another situation in which ketone bodies tend to limit their own production rate. It is clear, however, that in this case, the feed-back control mechanism is insufficient to maintain ketone bodies at concentrations compatible with life.

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