

# Physiologic Mechanisms in the Development of Starvation Ketosis in Man

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## SUMMARY

The present study was undertaken to determine whether alterations in ketone body utilization and hepatic production, independent of the FFA load, were also involved in the development of fasting ketosis.

Plasma  $\beta$ -OH butyric acid ( $\beta$ -OHB) increased to 2.5-4.5 mM and plasma FFA to 1,000-1,400  $\mu$ Eq/L. in normal weight individuals after five to seven days' starvation and in obese subjects after ten to fourteen days' fasting. Acute elevations of the plasma FFA > 1,500  $\mu$ Eq/L. for sixty minutes in fed normal weight and obese subjects with a fat meal-heparin regimen resulted in peak elevations of plasma  $\beta$ -OHB (0.25-0.45mM), only 10 per cent of that seen during fasting. When plasma FFA were lowered acutely during fasting with the antilipolytic agent Pyrazole to control levels (400-600  $\mu$ Eq/L.), plasma  $\beta$ -OHB decreased  $35 \pm 5$  per cent. Comparable lowering of plasma FFA in normal weight or obese starved subjects given dexamethasone to maintain elevated fasting plasma insulin levels resulted in an  $87 \pm 3$  per cent decrease in plasma  $\beta$ -OHB. Similar studies in obese fasted subjects pretreated with an intravenous infusion of insulin (1.0 U/hr. for eight hours) before receiving Pyrazole resulted in a  $65 \pm 5$  per cent decrease in plasma  $\beta$ -OHB.

Plasma  $\beta$ -OHB half-life, determined after injections of 12 gm.  $\beta$ -OHB, increased significantly during fasting ( $110 \pm 15$  minutes) and was decreased when the fasting subjects were maintained on dexamethasone ( $65 \pm 7$  minutes).

These studies indicate that accelerated hepatic ketogenesis during starvation is a result of both enhanced activity of the enzymatic system(s) involved in ketone body production as well as an increased FFA load. The increase in plasma  $\beta$ -OHB during fasting reflects not only an accelerated rate of hepatic ketogenesis but also an impairment of peripheral utilization, both processes apparently being sensitive to insulin. *DIABETES* 24:10-16, January, 1975.

The progressive rise in the circulating levels of beta-hydroxybutyric acid ( $\beta$ -OHB) and acetoacetic acid during the course of prolonged fasting has gener-

ally been attributed to a sequence of events represented by an accelerated rate of adipose tissue lipolysis, a greater fatty acid load presented to the liver and a consequent increase in hepatic ketone body production.<sup>1,2</sup> Since a decrease in plasma insulin to low but consistently detectable levels is a characteristic feature of the fasted state in all species thus far examined,<sup>3</sup> and the hormone is a known potent inhibitor of adipose tissue lipolysis,<sup>4</sup> insulin is assumed to be the primary humoral determinant regulating the development of ketosis. Recently, evidence has accumulated which indicates that insulin also influences markedly the activity of the hepatic enzyme system(s) involved in ketogenesis<sup>5,6</sup> and stimulates the utilization of ketones by peripheral tissues.<sup>6-8</sup> The present studies were undertaken, therefore, to determine the significance of changes in the rates of adipose tissue lipolysis, hepatic ketogenesis and ketone body utilization in the development of starvation ketosis in man.

## METHODS AND MATERIALS

### Experimental Procedures

Acute elevations in plasma free fatty acid levels in overnight fasted individuals were induced by feeding a 60 gm. fat meal consisting of 60 gm. corn oil, 60 ml. water, 15 gm. egg white, 6 ml. vanilla extract, 0.5 gm. salt and 0.4 ml. sodium cyclamate, followed in three hours by the intravenous administration of 5,000 units of heparin.<sup>9</sup> These studies were performed on nine obese individuals and three nonobese subjects; results in the latter group did not differ significantly from observations in the obese subjects so that all results were pooled for purposes of presentation.

To assess the effect of an abrupt decrease in plasma FFA on blood  $\beta$ -OHB levels, normal weight and obese subjects were fasted for one and two weeks, respectively, and then given orally 30 mg. of Pyrazole

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(5-methylpyrazole-3-carboxylic acid\*), a potent antilipolytic drug.<sup>10,11</sup> Blood samples were obtained at frequent intervals for measurement of FFA, glucose, insulin and  $\beta$ -OHB. Initially, acetoacetic acid levels were also determined, but since no significant differences in the ratio of  $\beta$ -OHB/AcAc were noted during the acute experimental period, only blood  $\beta$ -OHB was measured as an index of blood ketones.

The half-life ( $t_{1/2}$ ) of blood  $\beta$ -OHB was determined by injecting intravenously 12 gm. of DL-sodium  $\beta$ -OHB dissolved in 50 ml. normal saline over a two-minute period and obtaining frequent samples for measurement of blood  $\beta$ -OHB over the subsequent two hours. The slope of the blood  $\beta$ -OHB disappearance curve plotted as a semilogarithmic function was taken as the rate of utilization (K) and expressed as per cent disappearance per minute.

#### Analytical Procedure

Plasma glucose was determined by the Auto-Analyzer ferricyanide method,<sup>12</sup> and free fatty acids were measured by the colorimetric method of Duncombe<sup>13</sup> using the Dole extraction procedure.<sup>14</sup> Insulin was determined by radioimmunoassay,<sup>15</sup> and blood  $\beta$ -OHB was measured by the enzymatic method of Williamson et al.<sup>16</sup>

## RESULTS

### I. Effect of acute elevations of plasma free fatty acids in nonfasted subjects on blood $\beta$ -OHB levels.

Acute elevations of plasma FFA were induced in seven volunteers after an overnight fast by the fat meal-heparin regimen (figure 1). Plasma FFA levels rose from baseline levels of  $510 \pm 65 \mu\text{Eq/L.}$  to  $815 \pm 96 \mu\text{Eq/L.}$  three hours after the fat meal, attained peak levels of  $2,300 \pm 210 \mu\text{Eq/L.}$  ten minutes after the administration of heparin and declined slowly over the following sixty minutes to levels of  $1,750 \pm 200 \mu\text{Eq/L.}$  Basal blood  $\beta$ -OHB levels ranged from 0.01 mM to 0.03 mM and increased modestly (0.03 mM—0.05 mM) three hours after ingestion of the fat meal. Associated with the rapid rise in plasma FFA following injection of heparin, blood  $\beta$ -OHB increased twenty to forty-fold to 0.4–0.5 mM within ten minutes and remained at these levels for the following sixty minutes. Although the plasma FFA levels produced by the fat meal-heparin regimen (1,800–2,400  $\mu\text{Eq/L.}$ ) were nearly twice those observed routinely in subjects fasted for seven to fourteen days (1,000–1,300

\*Kindly supplied by Dr. K. Gundersen, Upjohn Company, Kalamazoo, Michigan.

### EFFECT OF ACUTE ELEVATION OF PLASMA FFA ON SERUM KETONE LEVELS AFTER OVERNIGHT FAST

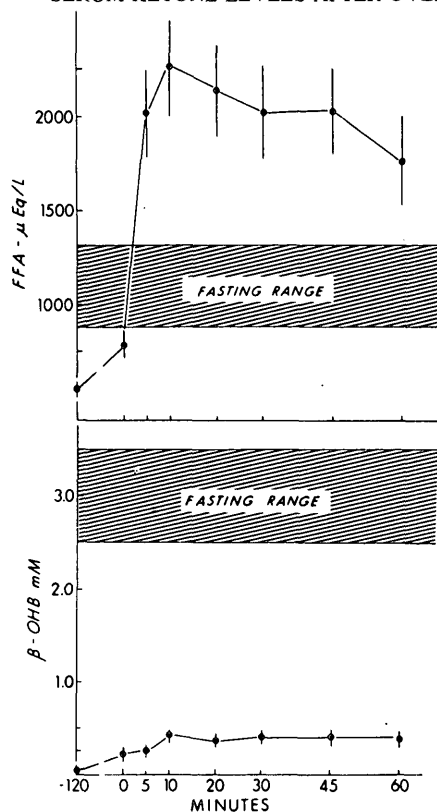


FIG. 1. The effect of acute elevations of the plasma FFA with the fat meal-heparin regimen on the blood  $\beta$ -OHB levels of normal and obese individuals after an overnight fast. All values represent the mean  $\pm$  S.E.M. of seven studies. Cross-hatched areas represent the mean  $\pm$  2 S.D. of the values seen after ten to fourteen days' starvation.

$\mu\text{Eq/L.}$ ), the ketone levels ( $0.42 \pm 0.06$  mmoles/L.) in this overnight fasted group were only 10 to 15 per cent of those observed during prolonged starvation (2.5–4.5 mM). No change in plasma insulin or glucose was observed in response to the acute elevation of plasma FFA, thus demonstrating that neither high FFA nor  $\beta$ -OHB levels in man constitute a physiologically significant insulinogenic stimulus.

### II. Effect of dexamethasone on plasma FFA and blood $\beta$ -OHB levels in fasting subjects.

Since starvation is characterized by a progressive decrease in insulin secretion,<sup>3</sup> studies were undertaken to evaluate the significance of this factor in the development of starvation ketosis. It had been shown previously in our laboratory<sup>17</sup> that the oral administration of glucocorticoids, such as Dexamethasone, produces a significant increase in the basal plasma insulin level. Seven subjects were therefore given Dexamethasone, 2 mg. orally every eight hours through-

out the course of a seven to fourteen day fast. Blood  $\beta$ -OHB levels in the steroid treated subjects ( $1.40 \pm 0.18$  mM) were 50 per cent lower than the ketone levels observed in the nontreated individuals ( $2.73 \pm 0.14$  mM) despite virtually identical levels of plasma FFA in both groups (figure 2). Basal insulin levels were significantly greater in the steroid treated subjects ( $28 \pm 4 \mu\text{U/ml.}$ ) than in the nontreated group ( $16 \pm 2 \mu\text{U/ml.}$ ) and approached the levels ( $35 \pm 6 \mu\text{U/ml.}$ ) observed routinely in nonfasted obese individuals.

EFFECT OF DEXAMETHASONE ON PLASMA INSULIN DURING PROLONGED FASTING

|                 |                              |                 |
|-----------------|------------------------------|-----------------|
| $1093 \pm 98$   | FFA ( $\mu\text{Eq/L}$ )     | $1044 \pm 68$   |
| $16 \pm 2$      | Insulin ( $\mu\text{U/ml}$ ) | $28 \pm 4$      |
| $2.73 \pm 0.14$ | $\beta$ -OHB (mM)            | $1.40 \pm 0.18$ |

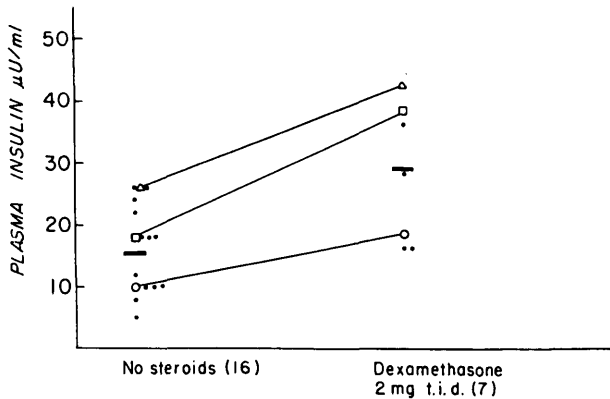


FIG. 2. Effect of dexamethasone (6 mg. daily) on the plasma insulin, FFA and blood  $\beta$ -OHB levels in obese individuals starved seven to fourteen days. Bars represent mean values.  $\Delta$ ,  $\circ$ ,  $\square$  represent three patients studied after ten days' starvation with and without receiving dexamethasone.

III. Effect of an acute decrease in plasma FFA on blood  $\beta$ -OHB.

A single 30 mg. dose of Pyrazole was given orally to subjects fasted seven to fourteen days to determine the effect of an acute lowering of the plasma FFA on blood ketones (figure 3). The plasma FFA decreased  $39 \pm 9$  per cent over a two-hour period (control level  $1,039 \pm 93 \mu\text{Eq/L.}$ ; nadir  $624 \pm 70 \mu\text{Eq/L.}$ ) with a corresponding  $35 \pm 6$  per cent decrease in blood  $\beta$ -OHB (control level  $2.73 \pm 0.14$  mM—nadir  $1.76 \pm 0.18$  mM). No significant change in plasma glucose or insulin levels was observed in response to Pyrazole. In contrast, fasting subjects receiving Dexamethasone displayed a much greater reduction ( $87 \pm 3$  per cent) in the blood  $\beta$ -OHB level (baseline  $1.40 \pm 0.18$  mM; nadir— $0.19 \pm 0.04$  mM) despite simi-

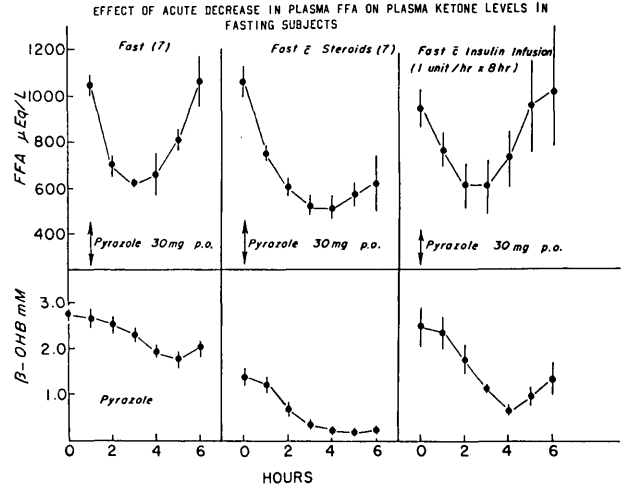


FIG. 3. Effect of an acute decrease in plasma FFA on blood  $\beta$ -OHB levels in seventeen to fourteen-day starved patients with and without prior treatment with oral dexamethasone (6 mg. daily) or insulin infusion (1 unit/hr. x 8 hours). Each point represents mean  $\pm$  S.E.M. of seven studies.

lar changes in the plasma FFA (baseline  $1,062 \pm 68 \mu\text{Eq/L.}$ ; nadir— $516 \pm 50 \mu\text{Eq/L.}$ ).

IV. Effect of a prolonged insulin infusion on plasma FFA and blood  $\beta$ -OHB in fasted subjects and the response to, Pyrazole.

Subjects fasted seven to fourteen days were given an intravenous infusion of 1.0 to 1.5 units of crystalline insulin per hour for eight hours (figure 4). This dose of hormone increased plasma insulin levels to the range of  $30$ - $40 \mu\text{U/ml.}$  within one hour and maintained these levels for the duration of the infusion. Decreases in plasma glucose were observed routinely within the initial 60 to 120 minutes of infusion, but none of these patients noted any symptoms referable to hypoglycemia even though occasional values in the range of 40 to 50 mg. per cent were observed between the sixth and eighth hour of the infusion. The mean plasma FFA and blood  $\beta$ -OHB levels did not exhibit any significant fall during the insulin infusion, although modest fluctuations were observed. Acute lowering of the plasma FFA with Pyrazole after stopping the insulin infusion resulted in a significantly greater fall ( $71 \pm 7$  per cent) in blood  $\beta$ -OHB than was noted in control subjects ( $35 \pm 6$  per cent) who did not receive insulin (figure 3).

V. Effect of a prolonged decrease in plasma FFA on blood  $\beta$ -OHB in fasted subjects.

To determine the effect of a prolonged decrease in plasma FFA on blood  $\beta$ -OHB, 40 mg. Pyrazole was

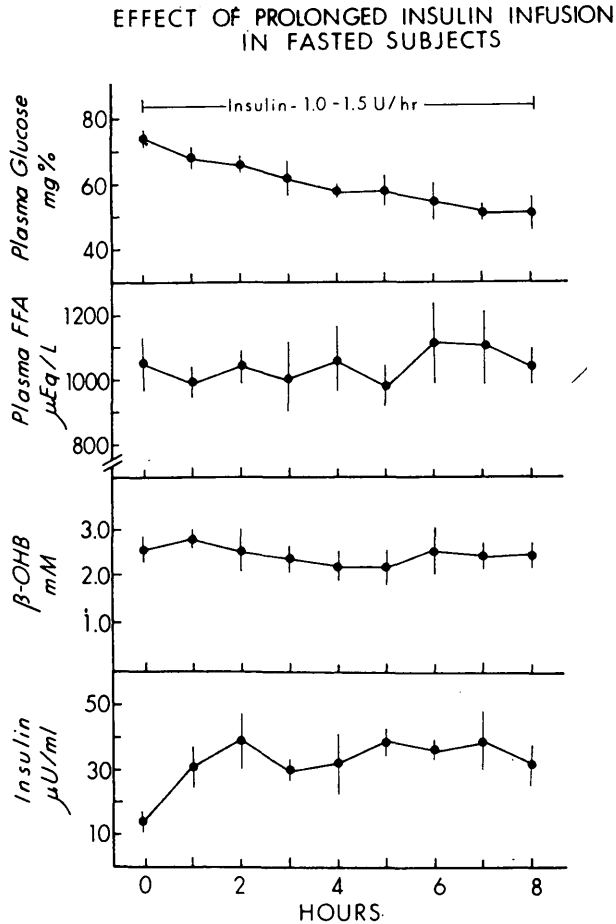


FIG. 4. Effect of infusion of 1.0-1.5 units of insulin per hour for eight hours on plasma glucose, FFA, insulin and blood  $\beta$ -OHB levels in ten to fourteen-day fasted obese subjects. Each value represents mean  $\pm$  S.E.M. of seven studies.

given orally every two and one-half hours for three doses (figure 5). The plasma FFA fell from initial levels of  $1,168 \pm 84 \mu\text{Eq/L}$ . to  $459 \pm 38 \mu\text{Eq/L}$ . by the third hour and were maintained at 40 to 50 per cent of control levels for six hours. As shown in figure 5, blood  $\beta$ -OHB continued to fall as long as the plasma FFA was decreased, but the maximum nadir ( $0.49 \pm 0.06 \text{ mM}$ ) reached after six hours was still significantly greater ( $p < 0.01$ ) than the level observed in steroid treated fasted subjects ( $0.19 \pm 0.04 \text{ mM}$ ) after two hours of FFA suppression. Furthermore, the rate of fall of blood  $\beta$ -OHB was also significantly faster in the steroid treated group despite comparable decreases in plasma FFA (figure 6).

#### VI. Effect of fasting and dexamethasone on the interrelationships between plasma FFA, insulin and blood $\beta$ -OHB levels.

Blood  $\beta$ -OHB levels for any given plasma FFA

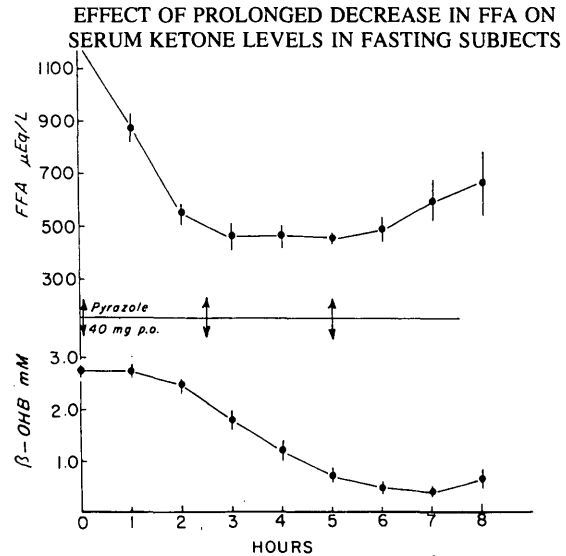


FIG. 5. Effect of a prolonged decrease in plasma FFA on blood  $\beta$ -OHB levels in seven subjects fasted ten to fourteen days. Pyrazole, 40 mg. per os was given in repeated doses at times indicated by arrows. Each value represents mean  $\pm$  S.E.M.

level after an overnight fast, prolonged fast or prolonged fast with steroid treatment are shown in figure 7. After an overnight fast, blood  $\beta$ -OHB levels rose to a maximum in the range of 0.4 to 0.5 mM when the plasma FFA level reached 1,200  $\mu\text{Eq/L}$ . and did not increase further as the plasma FFA was raised. This is in contrast to fasting subjects who demonstrated a progressive rise in blood  $\beta$ -OHB as the plasma FFA was increased. It is also apparent that fasting subjects receiving dexamethasone exhibited a lower blood  $\beta$ -OHB level for a given plasma FFA level than nonsteroid treated fasting subjects.

The relationship between plasma FFA, blood  $\beta$ -OHB and plasma insulin in prolonged fasting subjects is shown in figure 8. Although there is a significant inverse correlation between the plasma insulin and FFA level, the inverse correlation between plasma insulin and blood  $\beta$ -OHB is even more impressive, indicating that the antiketogenic effect of insulin involves mechanism(s) other than solely the antilipolytic activity of the hormone.

#### VII. Effect of fasting on blood $\beta$ -OHB half life.

The disappearance rate of  $\beta$ -OHB was determined in the same seven subjects after an overnight fast, a ten-day fast, and a ten-day fast while receiving dexamethasone (table 1). Following an overnight fast, the blood  $\beta$ -OHB half-life was  $23 \pm 2$  minutes. Starvation markedly prolonged the blood  $t_{1/2}$  to  $110 \pm 15$  minutes, but treatment with dexamethasone partially restored the half-life ( $65 \pm 7$  minutes) toward normal.

EFFECT OF REDUCTION OF FFA ON SERUM KETONE LEVELS IN FASTING SUBJECTS

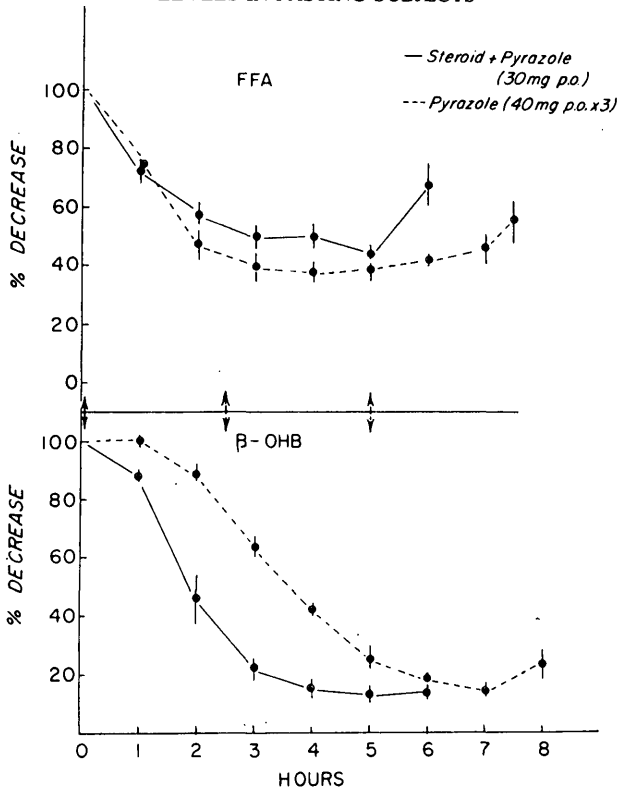


FIG. 6. Comparison of the effect of a comparably prolonged reduction of plasma FFA on blood  $\beta$ -OHB levels in ten to fourteen-day fasted subjects with and without prior treatment with dexamethasone (6 mg. daily). All values represent mean  $\pm$  S.E.M. of seven studies. Steroid treated patients received one dose of Pyrazole (30 mg. orally), whereas nonsteroid group received 40 mg. Pyrazole in repeated doses indicated by arrows.

The peak blood  $\beta$ -OHB levels attained five minutes after injection of 12 gm. of DL-sodium  $\beta$ -OHB were 4.7 to 4.9 mM in prolonged starvation, 3.8 to 4.2

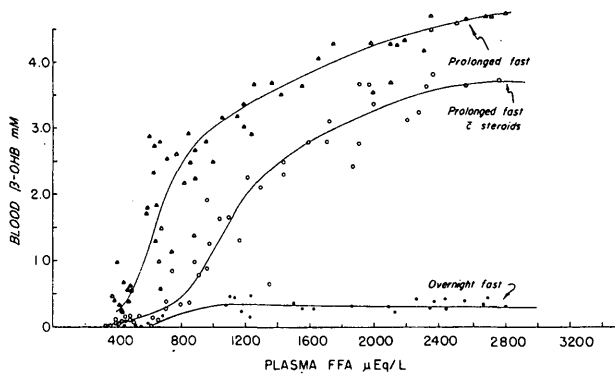


FIG. 7. Relationship of blood  $\beta$ -OHB levels and plasma FFA in overnight fasted, prolonged fasted (seven to fourteen days) and prolonged fasted-dexamethasone treated subjects.

RELATIONSHIP BETWEEN PLASMA INSULIN, FFA AND SERUM KETONES AFTER PROLONGED FAST

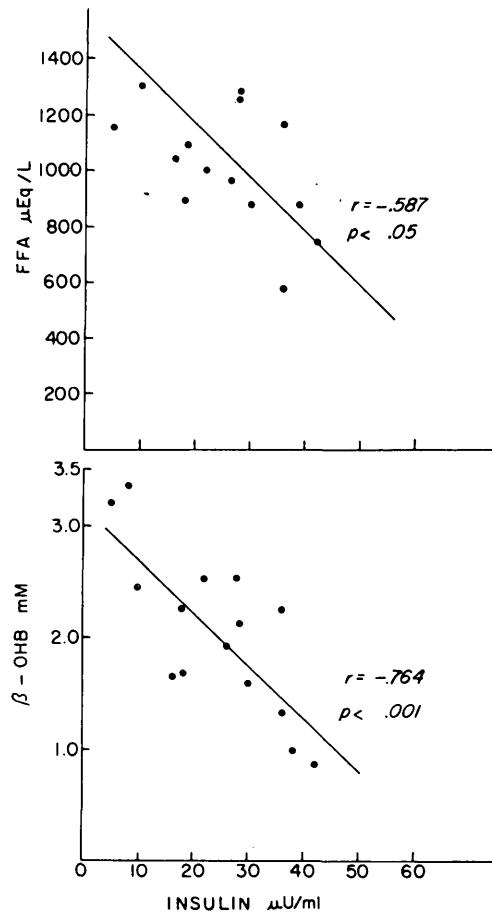


FIG. 8. Relationship of plasma FFA, blood  $\beta$ -OHB and plasma insulin levels in prolonged fasted (seven to fourteen days) individuals.

mM in the prolonged starvation-dexamethasone group and 1.8 to 2.3 mM in the fed state. No significant change in either plasma glucose or insulin was noted during the course of these studies.

TABLE 1  
Effect of prolonged fasting (ten to fourteen days) on blood  $\beta$ -OHB half-life

| Condition                       | Blood half-life | K                   |
|---------------------------------|-----------------|---------------------|
|                                 | minutes         | % $\text{min}^{-1}$ |
| Overnight fast                  | 23 $\pm$ 5      | 3.13 $\pm$ 0.71     |
| Prolonged fast                  | 110 $\pm$ 15    | 0.70 $\pm$ 0.07     |
| Prolonged fast + Dexamethasone* | 65 $\pm$ 7      | 1.11 $\pm$ 0.11     |

\*2 mg. every eight hours for duration of fast.

## DISCUSSION

Current concepts of the events leading to starvation ketosis assign a central role to an accelerated rate of adipose tissue lipolysis and its consequent increase in plasma FFA. Some relationship does exist between plasma FFA and blood  $\beta$ -OHB levels, for any alteration in plasma FFA is paralleled by a concomitant change in blood ketone levels (figure 7). However, the present studies clearly indicate that factors other than the plasma FFA level also contribute significantly to the development of starvation ketosis. For example, if the plasma FFA level is the only determinant, then the blood ketone values seen in overnight fasted subjects, in whom the plasma FFA are increased to twice the level observed during fasting by the fat meal-heparin regimen, should have been comparably elevated. Instead, their blood  $\beta$ -OHB levels rose to only 10 to 15 per cent of the values observed during prolonged fasting (figure 1). Furthermore, blood  $\beta$ -OHB and acetocetate continue to increase during prolonged fasting even though the plasma FFA level has reached a plateau.

Further evidence that factors other than adipose tissue lipolysis contribute to the development of starvation ketosis was obtained in the studies with dexamethasone. Despite virtually identical levels of plasma FFA, blood  $\beta$ -OHB levels in steroid treated fasted subjects were only half those of the nonsteroid treated group even after a two-week fast. Furthermore, when the plasma FFA level was lowered acutely with a single dose of Pyrazole, the rate of decrease of blood  $\beta$ -OHB in steroid treated subjects was significantly greater than in nonsteroid treated individuals. These results and the twofold increase in basal plasma insulin resulting from dexamethasone ingestion support the concept that insulin mediates the antiketogenic effect of glucocorticoids, but that this effect cannot be attributed exclusively to the antilipolytic action of insulin.

The experimental technics used in these studies do not permit the measurement of absolute rates of ketogenesis; however, some estimate of the relative rates of ketogenesis under different experimental conditions can be inferred by examining the relationship between the plasma FFA and the corresponding  $\beta$ -OHB levels (figure 7). In the prolonged fasted subject, the blood  $\beta$ -OHB level was ten to fifteen times greater than that attained in the overnight fasted individual whose plasma FFA concentration was increased acutely to that seen during extended starvation (i.e. 1,000-1,600  $\mu$ Eq/L.). Since the steady-state level of

blood ketones reflects their relative rates of production versus utilization, it is highly unlikely that changes of such magnitude could be accounted for exclusively by alterations in rates of utilization. Furthermore, considerable evidence has now accumulated from both *in vitro*<sup>5</sup> and *in vivo* studies<sup>6</sup> indicating that insulin markedly influences hepatic ketogenesis. Foster<sup>5</sup> has shown that insulin rapidly depresses the accelerated rate of hepatic ketogenesis seen in the starved rat. Our studies support the concept that insulin modulates hepatic ketogenesis, but they also indicate that the response in man differs temporally from that observed in the rat. Thus, infusions of hormone sufficient to restore plasma insulin to levels (i.e. 30-40  $\mu$ U/ml.) routinely observed under basal conditions in the obese human, failed to cause a significant fall in blood  $\beta$ -OHB or plasma FFA over an eight-hour period. A significant fall in plasma glucose was noted within sixty to ninety minutes, however, and suggests that the hormone was affecting hepatic gluconeogenesis before detectable changes were noted in hepatic ketogenesis. Similar findings following the infusion of minimal amounts of insulin (20 U/24 hrs.) into fasted obese subjects have recently been reported in preliminary form by Aoki et al. (see Cahill—Ref. 18). These workers noted that increasing the ambient plasma insulin level in fasted obese subjects to 15  $\mu$ U/ml. resulted in a progressive fall in blood glucose, plasma alanine and glutamine but no significant change in the plasma FFA or blood acetoacetic or  $\beta$ -OHB levels. Since gluconeogenic substrate availability appears to be the rate-limiting event regulating hepatic gluconeogenesis in fasted man,<sup>19</sup> our data support the concept proposed by Cahill<sup>18</sup> that amino acid efflux from skeletal muscle is a more sensitive index of insulin action in the fasted state than are either the antilipolytic or antiketogenic actions of the hormone. The prolonged infusion of insulin does exert some antiketogenic effect, since acutely lowering the plasma FFA with Pyrazole in fasted subjects who had been infused with insulin for eight hours resulted in a significantly greater reduction in blood  $\beta$ -OHB than was produced by a similar reduction in plasma FFA in fasted subjects not receiving insulin (figure 3). This study does not permit a clear cut distinction to be made between an effect of insulin on hepatic ketogenesis versus ketone utilization to account for this finding.

To explore the possibility that insulin affects ketone body utilization, the blood half-life ( $t_{1/2}$ ) of  $\beta^1$ -OHB was determined in overnight fasted, prolonged fasted and prolonged fasted-glucocorticoid treated subjects

by following the disappearance of a large bolus of  $\beta$ -OHB injected intravenously. Prolonged fasting markedly increased the blood  $\beta$ -OHB  $t_{\frac{1}{2}}$  (i.e.  $110 \pm 15$  minutes versus overnight fasted subjects  $23 \pm 5$  minutes) and glucocorticoids reduced this rate (i.e.  $65 \pm 7$  minutes) toward normal. These studies are subject to the criticism that the initial ketone pool size was considerably larger in the fasted groups and may have contributed, in part, to the differences observed in the plasma half-life of blood  $\beta$ -OHB. However, it should be noted that the differences in the peak blood  $\beta$ -OHB levels (i.e. five minutes after injection of the ketone) were not proportional to the changes observed in blood  $\beta$ -OHB  $t_{\frac{1}{2}}$ . Thus, the peak blood  $\beta$ -OHB level in prolonged fasting was 2.0 to 2.5 fold greater than that of the overnight fasted group, but the blood  $\beta$ -OHB  $t_{\frac{1}{2}}$  was more than five times greater. Correspondingly, the peak blood  $\beta$ -OHB of the fasted group was only 15 to 20 per cent greater than the level of the fasted-steroid treated group, but the blood  $\beta$ -OHB  $t_{\frac{1}{2}}$  was twice as long. These results are consistent with the thesis that the peripheral utilization of ketones is impaired after prolonged fasting and agree with the findings reported in dogs by Balasse and Havel,<sup>6</sup> and in man by Owen and Reichard.<sup>20</sup> They further support the suggestion that insulin accelerates the metabolism of these metabolites by insulin.<sup>7,8</sup>

Our studies indicate that the primary factor leading to the development of starvation ketosis is the insulinopenia that characteristically develops in the fasting state. This hormone deficiency results not only in an accelerated rate of adipose tissue lipolysis, but in a primary change in the activity of the hepatic ketogenic enzymatic system and an impairment in the mechanisms responsible for the peripheral utilization of ketones. It is all three of these events acting in concert which lead to the accumulation of blood ketones during starvation.

#### ACKNOWLEDGMENT

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