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*J Clin Invest.* 1970;49(9):1685-1693. <https://doi.org/10.1172/JCI106386>.

### Research Article

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Infusion of mannoheptulose with anti-insulin serum increased plasma glucose, FFA, and ketone body concentrations and decreased the specific activity of plasma FFA. Injection of insulin (20 mU i.v.) decreased almost simultaneously plasma glucose, FFA, and ketone body concentrations and increased the specific activity of FFA, but it did not affect the plasma concentration of FFA-<sup>3</sup>H. The findings indicate that insulin deprivation increased and insulin injection decreased the release of FFA from body tissues in fasting rats.

The plasma FFA concentration in fasting rats was increased by infusing chylomicrons and heparin, but this had very little effect on either plasma ketone body or glucose concentrations. Insulin injection (20 mU i.v.) lowered the plasma ketone body concentration in these animals. Studies using β-hydroxybutyrate-<sup>14</sup>C showed that insulin (50 mU i.v.) decreased ketogenesis in the presence of a sustained high plasma FFA concentration and had no effect on uptake of circulating ketone bodies.

The results [...]

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# Effect of Insulin and Acute Diabetes on Plasma FFA and Ketone Bodies in the Fasting Rat

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**ABSTRACT** The metabolism of FFA and ketone bodies was studied in fasted rats by infusing at a constant rate tracer amounts of FFA-<sup>3</sup>H,  $\beta$ -hydroxybutyrate-<sup>14</sup>C or acetoacetate-<sup>14</sup>C for periods up to 2 hr. Blood that was removed for analyses was replaced by continuous transfusion. The rates of turnover of FFA,  $\beta$ -hydroxybutyrate, and acetoacetate in rats fasted for 2 days were, respectively, 3.2, 5.6, and 2.5  $\mu$ moles/100 g body weight per min.

Infusion of mannoheptulose with anti-insulin serum increased plasma glucose, FFA, and ketone body concentrations and decreased the specific activity of plasma FFA. Injection of insulin (20 mU i.v.) decreased almost simultaneously plasma glucose, FFA, and ketone body concentrations and increased the specific activity of FFA, but it did not affect the plasma concentration of FFA-<sup>3</sup>H. The findings indicate that insulin deprivation increased and insulin injection decreased the release of FFA from body tissues in fasting rats.

The plasma FFA concentration in fasting rats was increased by infusing chylomicrons and heparin, but this had very little effect on either plasma ketone body or glucose concentrations. Insulin injection (20 mU i.v.) lowered the plasma ketone body concentration in these animals. Studies using  $\beta$ -hydroxybutyrate-<sup>14</sup>C showed that insulin (50 mU i.v.) decreased ketogenesis in the presence of a sustained high plasma FFA concentration and had no effect on uptake of circulating ketone bodies.

The results indicate that plasma FFA concentration is not the sole determinant of plasma ketone body concentration and that insulin can suppress ketone body production through some means other than lowering plasma FFA concentration.

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Received for publication 23 January 1970 and in revised form 3 April 1970.

## INTRODUCTION

Hyperketonemia in fasting and diabetic subjects results from the overproduction of ketone bodies (acetoacetate and D(-)- $\beta$ -hydroxybutyrate) in the liver (1-5) and is associated with a high concentration of FFA in the plasma (6, 7). Ketogenesis can be accelerated in isolated, perfused livers of normal animals by increasing the concentration of FFA in the perfusing fluid (8, 9). Plasma ketone body and FFA concentrations in fasting and diabetic animals are both decreased when insulin is administered (6, 7). Thus, hyperketonemia in these states could be the result of elevated plasma FFA concentration, and the effect of insulin on plasma ketone body level could be secondary to its lowering of plasma FFA concentration. Hyperketonemia, however, does not develop in dogs when their plasma FFA concentration is increased by the infusion of FFA (10) or by the infusion of a triglyceride emulsion with heparin (11). Results of other studies have suggested that insulin may lower plasma ketone body concentration by increasing the utilization of ketone bodies by extrahepatic tissues (2, 12, 13).

The present experiments were designed to clarify the roles of insulin and plasma FFA concentration in the development and reversal of hyperketonemia in the rat. The effects of insulin and insulin lack (14, 15) on plasma FFA and ketone body concentrations were studied in rats given a constant infusion of either labeled FFA or labeled ketone bodies.

## METHODS

**Animals.** Female Charles River rats were fed ad lib. Purina Laboratory Chow (Ralston Purina Co., St. Louis, Mo.) for at least 1 wk and then fasted either for 1 day (18 hr) or 2 days (42 hr) before the experiment. The experimental animals weighed between 125 and 170 g after the fast.

**Operative procedure.** The experimental rat was anesthetized with sodium pentobarbital (4.5 mg/100 g body weight

given i.p.) and placed upon its back. For the purpose of infusing blood and other substances, the right saphenous vein was cannulated with a short piece (3 cm long) of PE-10 polyethylene tubing (Clay-Adams, Inc., New York) attached to a piece (10 cm long) of AWG-22 vinyl plastic tubing (Temflex; 3M Company, St. Paul, Minn.). Heparin, 250  $\mu\text{g}$  in 0.3 ml 0.85% NaCl solution, was then given i.v. to prevent clotting (6). About 20 min later, the right saphenous artery was cannulated with a piece (6–12 cm long) of PE-10 polyethylene tubing connected to a piece (6 cm long) of AWG-22 vinyl tubing for the collection of continuous blood samples. Blood flow from the cannula was adjusted to a rate of either 0.15 or 0.30 ml/min by changing the length of the PE-10 tubing. The volume of the arterial tubing was less than 0.03 ml.

The rectal temperature of the experimental rat was maintained between 37°C and 38°C by warming the animal when necessary with an infrared lamp. Small doses of sodium pentobarbital (0.05–0.1 mg/100 g body weight) were given i.v. as needed to maintain a light level of anesthesia throughout the experiment.

**Sampling.** Arterial blood was collected continuously for periods of 5 or 10 min throughout the experiment. Since heparin injected intravascularly releases lipoprotein lipase to the bloodstream (16), precautions were taken that would block the activity of this enzyme in the blood samples. It was found that hydrolysis of chylomicrons was blocked completely by keeping the samples at a temperature below 3°C. Hence, blood samples were collected and stored in ice-chilled graduated tubes and centrifuged at 3°C. Aliquots of plasma were taken within 2 hr for analyses.

Blood loss due to blood sampling was replaced immediately by a continuous transfusion of heparinized whole blood. The rate of transfusion was adjusted so that it equalled the rate of blood flow from the arterial cannula. Transfusion blood was obtained just before each experiment from 6 to 10 rats fasted the same length of time as the experimental rat. The blood donors were anesthetized with ether, and 5–7 ml of blood was drawn from the aorta of each rat into a syringe containing 250  $\mu\text{g}$  of heparin in 0.1 ml of 0.85% NaCl solution. The blood was pooled and stored on ice until used.

**Materials infused.** Oleic acid-9,10- $^3\text{H}$  with a specific activity of 3.0 Ci/mmole (Nuclear-Chicago, Chicago, Ill.; TRK 140, Batch 2) was purified by thin-layer chromatography and complexed with rat serum albumin as previously described (17). The oleate- $^3\text{H}$ -albumin complex was stored in a freezer and diluted with rat serum just before being used. It was infused through the venous cannula at a constant rate of 1.80  $\mu\text{Ci}$  (in 7.9  $\mu\text{l}$ ) per min starting 30 min after anesthetizing the rat and 10 min before the experiment. A priming dose of 3.0  $\mu\text{Ci}$  was given immediately before starting the infusion. The specific activity of FFA in the plasma reached a near constant level within 15 min after the infusion was begun.

Sodium acetoacetate-3- $^{14}\text{C}$  was prepared by hydrolyzing ethyl acetoacetate-3- $^{14}\text{C}$  (specific activity 6.0 mCi/mmole; New England Nuclear Corp., Boston, Mass.; Lot 297-13) with 0.04 M  $\text{Na}_2\text{CO}_3$  at 55°C for 1 hr. The product was washed three times with ethyl ether and was used the same day it was prepared. It was found by using enzymatic (18), chemical (18), and chromatographic (Dowex-1( $\text{HCO}_3^-$ )) analyses that the product, acetoacetate-3- $^{14}\text{C}$ , was 98% pure. The labeled acetoacetate was infused i.v. at a constant rate of 0.30  $\mu\text{Ci}$  (in 7.9  $\mu\text{l}$ ) per min, starting 20 min before the

experiment. A priming dose of 0.5  $\mu\text{Ci}$  was given immediately before the infusion was begun.

Sodium *n*(-)- $\beta$ -hydroxybutyrate-3- $^{14}\text{C}$  was prepared enzymatically by the method of Bates, Krebs, and Williamson (18) from ethyl acetoacetate-3- $^{14}\text{C}$  (specific activity 6.0 mCi/mmole; New England Nuclear Corp.; Lot 297-13). The residual acetoacetate was destroyed by heating with 1 N  $\text{H}_2\text{SO}_4$ , and the labeled  $\beta$ -hydroxybutyrate was separated from the incubation medium by extraction with ethyl ether and collected in 0.1 M  $\text{NaHCO}_3$ . The final product contained less than 2% of the  $^{14}\text{C}$  as acetone or acetoacetate on the basis of enzymatic and radiochemical assays. More than 98% of the  $^{14}\text{C}$  was in the form of  $\beta$ -hydroxybutyrate as shown by the recovery of radioactivity in the Hg-acetone complex after enzymatic conversion of  $\beta$ -hydroxybutyrate to acetoacetate (see below). The labeled  $\beta$ -hydroxybutyrate was infused i.v. at a constant rate of 0.30  $\mu\text{Ci}$  (in 10  $\mu\text{l}$ ) per min.

Crystalline insulin (glucagon-free) was supplied by Dr. Mary Root of Eli Lilly & Co., Indianapolis, Ind. Guinea pig anti-insulin serum which could bind 3.65 U of insulin per ml was prepared and supplied by Dr. Peter Wright, Department of Pharmacology, Indiana University Medical Center. *D*-Mannoheptulose was isolated and supplied by Dr. Nelson K. Richtmyer of the National Institutes of Health. Chylomicrons were isolated, as described earlier (19), from thoracic duct lymph of rats fed corn oil. The chylomicrons were suspended in 4% bovine serum albumin and were injected i.v. at a rate of 0.80 or 1.10  $\mu\text{moles}$  of triglyceride (in 7.9  $\mu\text{l}$ ) per min.

**Analytical methods.** FFA concentration in plasma was determined by a modification of the method of Dole and Meinertz (20); hexane was used instead of heptane, and FFA were titrated in ethyl alcohol to a Nile blue endpoint. Plasma was deproteinized with  $\text{Ba}(\text{OH})_2\text{-ZnSO}_4$  (21) and analyzed for total ketone bodies,  $\beta$ -hydroxybutyrate, and acetoacetate by the procedures of Chernick (22) or of Bates et al. (18), as indicated, and for glucose by the glucose oxidase method (Worthington Biochemical Corp., Freehold, N. J.).

Radioactivity in plasma FFA was determined as follows. The alcoholic solution of FFA after titration was diluted with an equal volume of 0.01 N NaOH and washed three times with hexane to remove neutral lipids. The aqueous-alcohol solution was then acidified with  $\text{H}_2\text{SO}_4$ , and FFA were extracted into hexane. An aliquot of the hexane extract was evaporated, dissolved in toluene containing 4.2% Liquifluor (New England Nuclear Corp.; Cat. No. NEF-903), and  $^3\text{H}$  content was measured in a Packard Tri-Carb liquid scintillation spectrometer.

$^{14}\text{C}$  in acetoacetate,  $\beta$ -hydroxybutyrate, and total ketone bodies was determined as follows. An aliquot of deproteinized plasma (21) was transferred to a 16  $\times$  150 screw-capped culture tube and mixed with carrier acetoacetate (50  $\mu\text{moles}$ ), carrier *D*, *L*- $\beta$ -hydroxybutyrate (5  $\mu\text{moles}$ ) and 1 ml 0.25 M  $\text{HgSO}_4$  in 4 N  $\text{H}_2\text{SO}_4$ . The tube was closed tightly with a Teflon-lined screw cap, heated for 60 min at 130°C (22), cooled in ice water, and centrifuged to precipitate the Hg-acetone complex formed from acetoacetate (18). The method for measuring  $^{14}\text{C}$  in the Hg-acetone complex is described below. The remaining supernatant was transferred to another 16  $\times$  150 mm tube and mixed with carrier acetoacetate (25  $\mu\text{moles}$ ),  $\text{K}_2\text{Cr}_2\text{O}_7$  (15  $\mu\text{moles}$  for oxidation of  $\beta$ -hydroxybutyrate), and 1 ml of the above  $\text{HgSO}_4\text{-H}_2\text{SO}_4$  solution. The tube was tightly capped, heated, cooled, and centrifuged, as described above, to produce a Hg-acetone

TABLE I  
*Plasma Glucose, Ketone Body, and FFA Concentrations in the Blood of Experimental Animals during the First 20 min of the Experiment and in Transfusion Blood\**

No. of days fasted	No. of experiments	Experimental animals			Transfusion blood†		
		Plasma glucose	Plasma ketone bodies	Plasma FFA	Plasma glucose	Plasma ketone bodies	Plasma FFA
1	10	5.8 ± 0.1	0.95 ± 0.08	0.70 ± 0.04	5.8 ± 0.2	0.80 ± 0.03	0.70 ± 0.03
2	12	5.8 ± 0.2	1.27 ± 0.07	0.66 ± 0.02	5.4 ± 0.2	1.26 ± 0.06	0.79 ± 0.02

\* Values are means ± SE. Total ketone bodies were measured by the chemical procedure (22).

† Plasma glucose, ketone body, and FFA concentrations in transfusion blood did not change significantly when the blood was kept at 0°C for 3 hr.

complex containing <sup>14</sup>C derived solely from β-hydroxybutyrate-<sup>14</sup>C. The <sup>14</sup>C in total ketone bodies was determined, as described by Bates et al. (18), in the sample used for enzymatic determination of acetoacetate and total ketone bodies; the aliquot was treated as described above for the determination of acetoacetate-<sup>14</sup>C to yield a Hg-acetone complex containing <sup>14</sup>C derived from both acetoacetate and β-hydroxybutyrate. The Hg-acetone complexes were washed with ice water, dissolved in 0.6 ml 2 N HCl and then mixed in screw-capped tubes with 15 ml of scintillation solution consisting of 24% Triton X-100 (Packard Instrument Co., Inc., Downers Grove, Ill.), 4% Liquifluor, and 72% toluene. The tubes were shaken, and the contents were transferred to counting vials for measurement of <sup>14</sup>C content in a liquid scintillation spectrometer. Standard solutions of acetoacetate-<sup>14</sup>C and β-hydroxybutyrate-<sup>14</sup>C added to plasma were analyzed at the same time, and corrections were made for recovery of radioactivity in the Hg-acetone complex. Generally 80–90% of the <sup>14</sup>C standards were recovered. The <sup>14</sup>C content of the total ketone body fraction agreed closely with the sum of the <sup>14</sup>C contents of the acetoacetate and β-hydroxybutyrate fractions.

## RESULTS

Plasma concentrations of glucose, ketone bodies, and FFA in the blood of experimental animals during the first 20 min (control period) are compared in Table I with plasma concentrations in pooled transfusion blood. The plasma ketone body concentration was higher in animals fasted 2 days than in those fasted 1 day, whereas the plasma glucose and FFA levels were similar. There were no significant differences between experimental animals and blood donors fasted the same length of time, except that plasma FFA concentration in rats fasted 2 days was lower in the experimental animals than in the blood donors. The plasma glucose, ketone body, and FFA concentrations in the control animals changed less than 20% in 90 min (Fig. 1).

When oleic acid-<sup>3</sup>H was infused, a priming dose was given and the infusion was begun 10 min before the experiment. The plasma concentration of FFA-<sup>3</sup>H in control animals fasted 1 and 2 days reached a nearly constant level within 10 min and then increased slightly

during the rest of the experiment, about 20% in 70 min (Figs. 2 and 3). The specific activity of plasma FFA, however, was nearly constant throughout the experiment (Figs. 2 and 3).

The rate of turnover of plasma FFA in control animals was calculated from the rate of infusion of oleic acid-<sup>3</sup>H and the specific activity of plasma FFA after

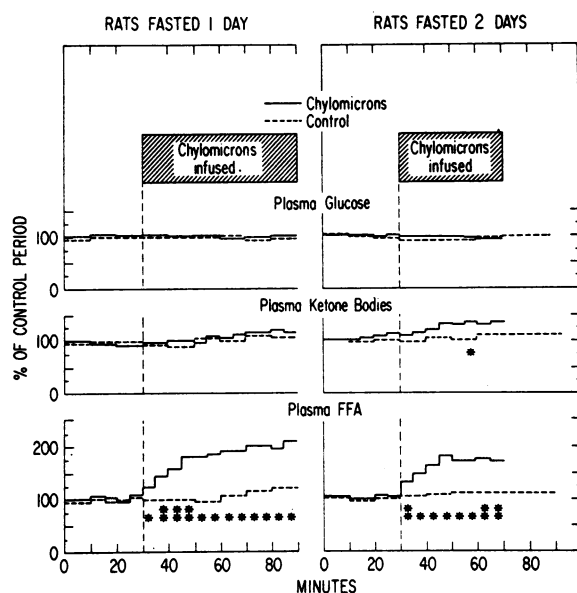


FIGURE 1 Effect of infusing chylomicrons on plasma glucose, ketone body, and FFA concentrations in rats fasted 1 and 2 days. The mean plasma glucose, ketone body (22), and FFA concentrations (mM) during the first 30 min were, respectively, as follows: 5.9 ± 0.2 (SEM), 1.02 ± 0.07, and 0.69 ± 0.02 in 1 day fasted controls; 5.7 ± 0.3, 0.91 ± 0.11, and 0.77 ± 0.07 in 1 day fasted rats infused with chylomicrons; 5.4 ± 0.2, 1.39 ± 0.13, and 0.64 ± 0.06 in 2-day fasted controls; and 5.7 ± 0.4, 1.36 ± 0.08, and 0.71 ± 0.03 in 2-day fasted rats infused with chylomicrons. There were three animals in each group. Statistically significant differences are indicated by either single ( $P < 0.05$ ) or double ( $P < 0.01$ ) asterisks.

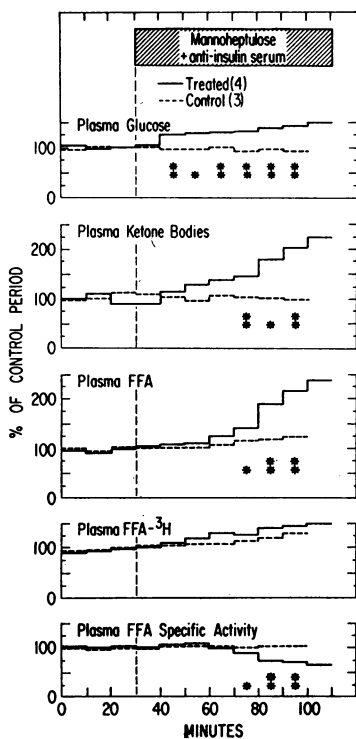


FIGURE 2 Effect of infusing mannoheptulose and anti-insulin serum on plasma glucose, ketone body (22), FFA, and FFA-<sup>3</sup>H concentrations, and plasma FFA specific activity in rats fasted 1 day. 10 min before the experiment, all rats were given a priming dose of oleic acid-<sup>3</sup>H and then a continuous infusion of oleic acid-<sup>3</sup>H throughout the experiment. The mean plasma glucose, ketone body, and FFA concentrations (mM) during the first 30 min were, respectively,  $5.9 \pm 0.2$ ,  $1.02 \pm 0.07$ , and  $0.69 \pm 0.02$  in the control, and  $6.1 \pm 0.1$ ,  $0.93 \pm 0.19$ , and  $0.65 \pm 0.09$  in the treated group. There were three animals in the control and four in the treated group. Statistically significant differences are indicated by either single ( $P < 0.05$ ) or double ( $P < 0.01$ ) asterisks.

60 min of infusion (23). The turnover rate was  $3.5 \pm 0.3$   $\mu$ moles/100 g body weight per min in rats fasted 1 day and  $3.2 \pm 0.3$  in rats fasted 2 days.

**Effect of acute insulin deprivation.** Acute insulin deprivation was induced in rats fasted 1 day by i.v. administration of D-mannoheptulose (MH) and guinea pig anti-insulin serum (AIS). The substances were given together because preliminary experiments showed that neither one, given alone, caused any change in plasma glucose, ketone bodies, or FFA in anesthetized animals receiving a continuous transfusion of normal blood. At 30 min after starting the experiment, 100 mg of MH (in 20% solution) and 1 ml of AIS were injected through the venous cannula in 1 min, and 200 mg of MH and 1.5 ml of AIS were then injected slowly during the remaining 80 min of the experiment.

The plasma glucose concentration increased 25% at 10 min after injection of MH-AIS, whereas the plasma FFA and ketone bodies did not increase significantly until after 40 min (Fig. 2). The maximal increment in glucose concentration, however, was only 50%, whereas that in ketone body and FFA concentrations was more than 100%. The plasma ketone body and FFA concentrations increased at nearly equal rates. There was also an increase in plasma FFA-<sup>3</sup>H concentration, but it was proportionally less than that in plasma FFA concentration. Consequently, the specific activity of plasma FFA decreased, indicating that the release of FFA from body tissues to blood plasma was accelerated by insulin deprivation (Fig. 2).

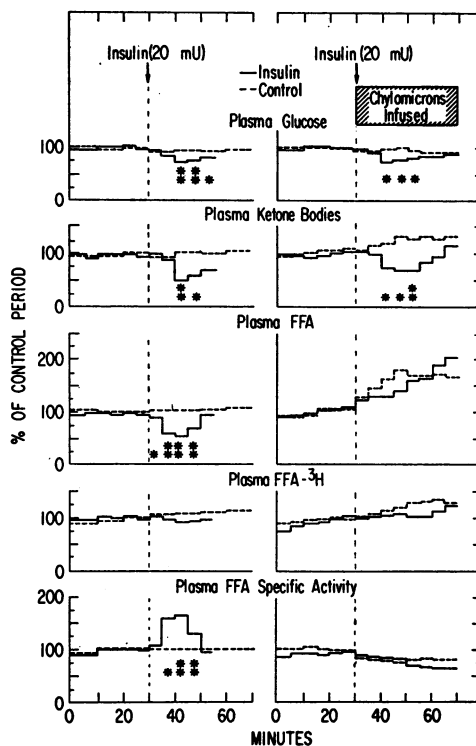


FIGURE 3 Effect of a single injection of insulin (20 mU) subcutaneously on plasma glucose, ketone body (22), FFA, and FFA-<sup>3</sup>H concentrations, and plasma FFA specific activity in rats fasted 2 days. Some of the rats were infused continuously with chylomicrons. All rats were given 10 min before the experiment a priming dose of oleic acid-<sup>3</sup>H and then a continuous infusion of oleic acid-<sup>3</sup>H throughout the experiment. The mean plasma glucose, ketone body, and FFA concentrations (mM) during the first 30 min were, respectively, as follows:  $5.4 \pm 0.2$ ,  $1.39 \pm 0.13$ , and  $0.64 \pm 0.06$  in controls;  $5.9 \pm 0.3$ ,  $1.22 \pm 0.23$ , and  $0.65 \pm 0.05$  in insulin-treated;  $5.7 \pm 0.4$ ,  $1.36 \pm 0.08$ , and  $0.71 \pm 0.03$  in chylomicron controls; and  $5.9 \pm 0.4$ ,  $1.16 \pm 0.10$ , and  $0.66 \pm 0.01$  in chylomicron insulin-treated group. There were three rats in each group. Statistically significant effects of insulin are indicated by either single ( $P < 0.05$ ) or double ( $P < 0.01$ ) asterisks.

*Effect of insulin.* The effect of a small dose of insulin (20 mU) on plasma glucose, ketone body, and FFA concentrations was studied in rats fasted for 2 days (Fig. 3). Infusion of labeled FFA was begun 10 min before the experiment and 40 min before the insulin was given i.v. Insulin decreased the plasma FFA concentration within 5 min and decreased glucose and ketone body concentrations within the next 10 min. The plasma FFA concentration returned to the preinjection level 20 min after the injection of insulin, whereas the plasma ketone body and glucose concentrations were still below control values at this time. The plasma FFA-<sup>3</sup>H concentration was not affected by insulin, whereas the plasma FFA specific activity was increased more than 50%, indicating that insulin decreased the release of FFA to the plasma.

*Effect of chylomicron infusion.* In the above experiments, all changes in plasma ketone body concentration in response to insulin administration and insulin lack were preceded or accompanied by change in plasma FFA concentration, suggesting that the changes in ketonemia were secondary to those in plasma FFA concentration. In order to test this possibility, plasma FFA concentration in fasting rats was increased by i.v. infusion of chylomicrons. The infused chylomicron triglycerides were hydrolyzed to FFA by lipoprotein lipase released to the bloodstream by heparin given with the transfusion. The chylomicrons were infused through the venous cannula at a rate of 0.8  $\mu$ moles of triglyceride per min.

Plasma FFA concentration in control rats fasted for 2 days was increased 30% within 10 min and 70% within 30 min after starting the infusion of chylomicrons (Fig. 1). Although the plasma glucose concentration was not changed, both plasma ketone body and FFA-<sup>3</sup>H concentrations were increased 30% (Figs. 1 and 3). The specific activity of plasma FFA was decreased 20%, indicating that addition of FFA to the plasma had been increased, presumably as the result of intravascular hydrolysis of infused chylomicron triglyceride. In 1 day fasted rats, the plasma FFA concentration was increased more than 80% within 40 min by the infusion of chylomicrons, but the plasma ketone body and glucose concentrations were not affected (Fig. 1). The specific activity of plasma FFA in this group was decreased 25%.

*Effect of insulin on animals infused with chylomicrons.* The effect of insulin administration on plasma ketone bodies in animals with high plasma FFA concentration was studied in rats fasted 2 days and infused with chylomicrons (Fig. 3). Intravenous injection of 20 mU of insulin significantly lowered plasma glucose and ketone body concentrations, but it did not affect significantly the plasma FFA concentration. The decrease in plasma ketone bodies occurred even in the presence of high

plasma FFA concentrations. There was no significant effect of insulin on plasma FFA-<sup>3</sup>H concentration or FFA specific activity in the animals infused with chylomicrons. The lowering effect of insulin on ketone body concentration in the presence of unchanged high plasma FFA concentrations suggests that insulin may either accelerate clearance of ketone bodies from the blood or inhibit ketogenesis directly.

*Studies with  $\beta$ -hydroxybutyrate-<sup>14</sup>C and acetoacetate-<sup>14</sup>C.* Whether the fall in plasma ketone body concentration in insulin-treated rats infused with chylomicrons was due to decreased formation or increased utilization of ketone bodies was studied in animals given a constant infusion of  $\beta$ -hydroxybutyrate-<sup>14</sup>C. Infusion of labeled  $\beta$ -hydroxybutyrate was begun 55 min before the injection of insulin and the start of the infusion of chylomicrons. The concentration of <sup>14</sup>C-labeled  $\beta$ -hydroxybutyrate and the specific activity of  $\beta$ -hydroxybutyrate in the plasma became constant about 20 min after starting the infusion of labeled ketone body and remained unchanged thereafter until 10 min after the injection of insulin (Fig. 4). The immediate appearance of acetoacetate-<sup>14</sup>C in the plasma indicated that  $\beta$ -hydroxybutyrate was rapidly converted to acetoacetate. The concentration of acetoacetate-<sup>14</sup>C and the specific activity of acetoacetate in plasma became constant about 20–30 min after starting the infusion of  $\beta$ -hydroxybutyrate-<sup>14</sup>C, but the specific activity was only 60% of that of  $\beta$ -hydroxybutyrate (Table II).

Insulin (50 mU) was injected i.v., and a constant infusion of chylomicrons (1.10  $\mu$ moles of triglycerides per min) was begun at 55 min after starting the infusion of labeled  $\beta$ -hydroxybutyrate. The plasma FFA concentration increased 58% during the first 5 min and continued to increase during the next 50 min to 200% above the control level. Plasma glucose concentration decreased 34% during the first 15 min after insulin and then gradually returned to the control level. The plasma concentrations of  $\beta$ -hydroxybutyrate and acetoacetate both decreased during the first 20 min after insulin, 49% and 27%, respectively, and then increased during the next 40 min to levels 50% above those before insulin was given. Plasma concentrations of  $\beta$ -hydroxybutyrate-<sup>14</sup>C and acetoacetate-<sup>14</sup>C did not change significantly after the injection of insulin. The specific activities of both ketone bodies increased significantly during the first 20 min after insulin and then decreased to values significantly below those before insulin was given. These findings indicate that insulin decreases ketogenesis in the presence of a high plasma FFA level, and that insulin under these conditions has no effect on the uptake of circulating ketone bodies in fasted rats.

Turnover of  $\beta$ -hydroxybutyrate during the control period was calculated from the rate of infusion of

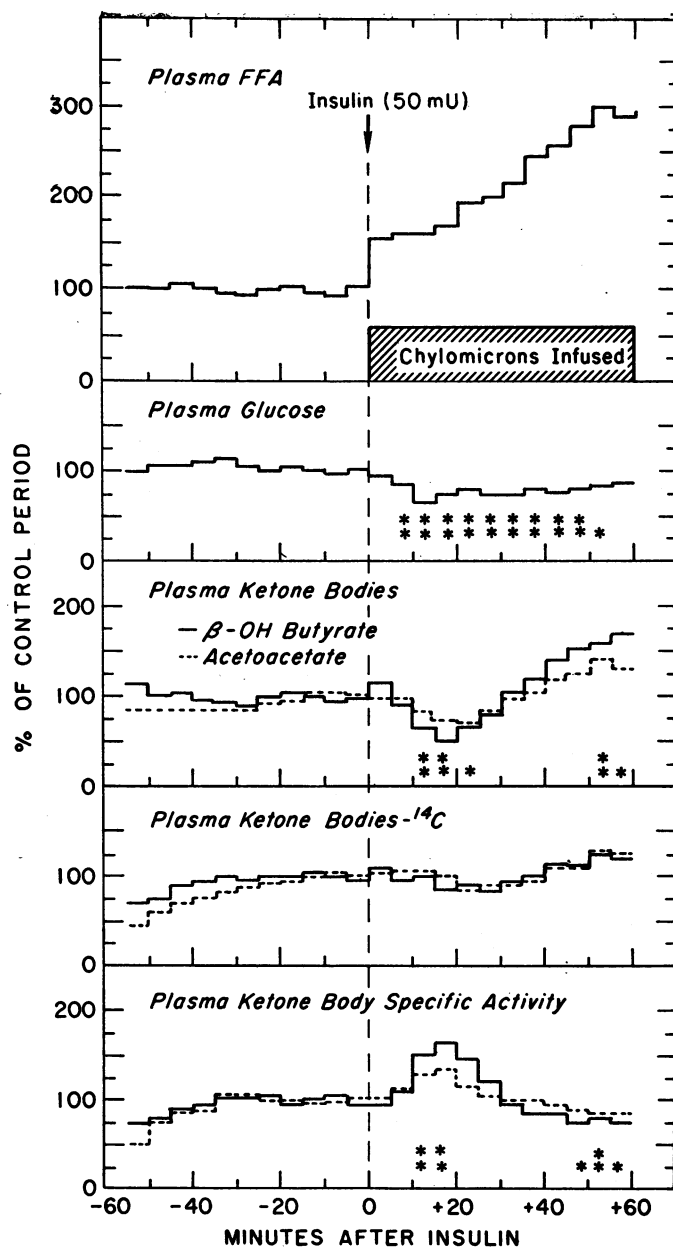


FIGURE 4 Effect of a single injection of insulin (50 mU given i.v.) on the plasma concentrations of FFA, glucose, ketone bodies (18), and ketone bodies- $^{14}\text{C}$ , and on the specific activity of plasma ketone bodies in four rats fasted 2 days and infused continuously with  $\beta$ -hydroxybutyrate- $^{14}\text{C}$  (started 50 min before insulin) and chylomicrons (started when insulin was given). The mean plasma FFA, glucose,  $\beta$ -hydroxybutyrate, and acetoacetate concentrations (mM) during the 30 min before insulin were, respectively,  $0.69 \pm 0.05$ ,  $6.3 \pm 0.4$ ,  $1.0 \pm 0.2$ , and  $0.7 \pm 0.1$ . The plasma concentrations of  $\beta$ -hydroxybutyrate- $^{14}\text{C}$  and acetoacetate- $^{14}\text{C}$  during the same time were, respectively,  $65 \pm 3$  and  $31 \pm 3 \times 10^8$  dpm/ml, and the specific activities were  $71 \pm 9$  and  $45 \pm 4 \times 10^8$  dpm/ $\mu\text{mole}$ . Statistically significant effects of insulin on plasma glucose,  $\beta$ -hydroxybutyrate, acetoacetate,  $\beta$ -hydroxybutyrate- $^{14}\text{C}$  and acetoacetate- $^{14}\text{C}$  concentrations and on specific activity of  $\beta$ -hydroxybutyrate and acetoacetate are indicated by either single ( $P < 0.05$ ) or double ( $P < 0.01$ ) asterisks.

TABLE II  
Concentration, Specific Activity, and Rate of Turnover of Plasma Ketone Bodies in Fasted Rats Infused with Either  $\beta$ -Hydroxybutyrate- $^{14}\text{C}$  or Acetoacetate- $^{14}\text{C}$ \*

Radioactive substance infused	No. of rats	$\beta$ -Hydroxybutyrate	Acetoacetate	Total ketone bodies
<i>Concentration (mM)</i>				
$\beta$ -Hydroxybutyrate	4	1.0 $\pm$ 0.2	0.7 $\pm$ 0.1†	1.7 $\pm$ 0.2‡
Acetoacetate	3	0.6 $\pm$ 0.005	0.5 $\pm$ 0.002§	1.1 $\pm$ 0.1§
Acetoacetate	1	0.6	0.5§	1.1§
<i>Specific activity (dpm <math>\times</math> 10<sup>-3</sup>/<math>\mu</math>mole)</i>				
$\beta$ -Hydroxybutyrate	4	71 $\pm$ 9	45 $\pm$ 4	60 $\pm$ 8
Acetoacetate	3	—	117 $\pm$ 21	—
Acetoacetate	1	<30	110	—
<i>Turnover (<math>\mu</math>moles/100 g body weight per min)</i>				
$\beta$ -Hydroxybutyrate	4	5.6 $\pm$ 0.8	—	[6.5 $\pm$ 0.7]
Acetoacetate	3	—	2.5 $\pm$ 0.3	—

\* All rats were fasted for 2 days before the experiment. D(—)- $\beta$ -Hydroxybutyrate- $^{14}\text{C}$  was infused at a constant rate of 0.30  $\mu\text{Ci}$  (in 11.0  $\mu\text{l}$ ) per min; the data presented were obtained during a steady-state period of 30 min that began 25 min after the start of the infusion (see Fig. 4). Acetoacetate- $^{14}\text{C}$  was infused at a constant rate of 0.30  $\mu\text{Ci}$  (in 7.9  $\mu\text{l}$ ) per min after giving a priming dose of 0.5  $\mu\text{Ci}$ ; the data were obtained during a steady-state period of 20 min that began 20 min after the start of the infusion.

† Acetoacetate and total ketone bodies were measured by enzymatic method of Bates et al. (18);  $\beta$ -hydroxybutyrate was determined by difference.

§ Acetoacetate and total ketone bodies were measured by chemical method of Chernick (22);  $\beta$ -hydroxybutyrate by difference.

|| The limitations of this estimate are described in the Discussion.

$\beta$ -hydroxybutyrate- $^{14}\text{C}$  and the average specific activity of  $\beta$ -hydroxybutyrate during the steady-state period of 30 min before insulin was injected (23). The rate of turnover of  $\beta$ -hydroxybutyrate in rats fasted for 2 days was 5.6  $\mu\text{moles}/100$  g body weight per min (Table II). The turnover of total ketone bodies was estimated from the rate of infusion of  $\beta$ -hydroxybutyrate- $^{14}\text{C}$  and the specific activity of total ketone bodies before insulin was injected; the estimate was 6.5  $\mu\text{moles}/100$  g body weight per min. The limitations of this estimate, however, are discussed below. Turnover of acetoacetate was not calculated in these animals because the rate of entry of acetoacetate- $^{14}\text{C}$  into the plasma was not known. Therefore, another group of rats was infused with acetoacetate- $^{14}\text{C}$  at a constant rate, and turnover of acetoacetate was calculated from the rate of infusion and the average specific activity of acetoacetate in the plasma during a steady-state period of 20 min that began 20 min after the start of the infusion. The rate of turnover of acetoacetate in rats fasted for 2 days was 2.5  $\mu\text{moles}/100$  g body weight per min (Table II). Plasma  $\beta$ -hydroxybutyrate became labeled when acetoacetate- $^{14}\text{C}$  was infused, but its specific activity was less than half of that of acetoacetate (Table II).

## DISCUSSION

The above studies show that insulin lowered the plasma FFA concentration in fasting rats by decreasing the re-

lease of FFA to the plasma, presumably from adipose tissue. Insulin also decreased the plasma ketone body concentration but at a slightly later time. Induction of acute insulin deprivation with mannoheptulose and anti-insulin serum had effects in the fasting rat opposite to those of insulin administration; it increased the release of FFA to the blood and increased the concentrations of FFA and ketone bodies in the plasma. These findings are consistent with the view that insulin decreases plasma ketone body levels by lowering the plasma FFA concentration through its inhibition of FFA release by adipose tissue.

If plasma FFA concentration were the sole determinant of plasma ketone body level, increasing plasma FFA concentration alone would cause hyperketonemia. However, increasing plasma FFA concentration by the infusion of chylomicrons and heparin had no effect on plasma ketone body concentration in rats fasted 1 day and only a slight effect in rats fasted 2 days. Similar observations have been made in dogs (10, 11). That other factors are involved in the development of hyperketonemia is also indicated by the difference in plasma ketone body concentration between rats fasted 1 day and those fasted 2 days even though their plasma FFA concentrations were similar (Table I).

Ketone body production by perfused rat liver is increased when the FFA concentration of the perfusing fluid is elevated (5, 24). However, the increment due



to the change in plasma FFA concentration is much less in livers of fed rats than in livers of fasted rats (5).<sup>1</sup> Assays of the enzymes involved in the formation of ketone bodies in the liver have shown that the enzyme activities are not increased by fasting (25). Ketogenesis in perfused livers of well-nourished diabetic rats is much greater than that of normal rats and is not affected appreciably by elevating the FFA concentration in the perfusing fluid (4, 5, 9). Additional evidence that plasma FFA concentration is not the sole determinant of ketogenesis is the recent observation that glucagon, which did not affect uptake of FFA by liver, increased the rate of ketogenesis in perfused liver of fed rats (24). The nature of the changes produced in the liver by fasting, diabetes, and glucagon that enhance ketogenesis requires further study.

Injection of insulin lowered the plasma ketone body concentration without changing the plasma FFA concentration in rats given a continuous infusion of chylomicrons and heparin (Fig. 3). This suggested that insulin might lower the plasma ketone body concentration either by decreasing ketone body production independently of the plasma FFA concentration or by enhancing ketone body utilization (2, 12, 13). Foster found in experiments with radioactive acetoacetate that insulin decreased the synthesis of acetoacetate in fasting rats *in vivo* (26). The possibility that this effect was mediated through a lowering of plasma FFA in his study was not ruled out. The above studies with  $\beta$ -hydroxybutyrate-<sup>14</sup>C in rats given a constant infusion of chylomicrons and heparin (Fig. 4) showed that insulin decreased the delivery of ketone bodies, both acetoacetate and  $\beta$ -hydroxybutyrate, to the plasma and that this occurred in the presence of a sustained elevated plasma FFA concentration. Insulin did not affect the rate of clearance of ketone bodies from the bloodstream. These observations indicate that insulin has a suppressing effect on ketogenesis, perhaps in the liver, independent of any effect on the plasma FFA concentration. Insulin, of course, can also decrease ketogenesis *in vivo* by suppressing the release of FFA from adipose tissue and, thus, lowering the plasma FFA concentration (6, 27). The effect of this on ketogenesis, however, may be small and delayed if the liver contains extra amounts of triglyceride as in diabetic animals (2).

Turnover of plasma ketone bodies was determined in rats by giving a continuous infusion of tracer amounts of either  $\beta$ -hydroxybutyrate-<sup>14</sup>C or acetoacetate-<sup>14</sup>C. The immediate appearance of acetoacetate-<sup>14</sup>C in plasma when  $\beta$ -hydroxybutyrate-<sup>14</sup>C was infused indicated that  $\beta$ -hydroxybutyrate was rapidly converted to acetoacetate (Fig. 4). Although the specific activities of the two

ketone bodies became constant within 30 min after the start of the infusion, they were not equal; the specific activity of acetoacetate was 60% of that of  $\beta$ -hydroxybutyrate (Table II). Plasma  $\beta$ -hydroxybutyrate also became labeled when acetoacetate-<sup>14</sup>C was infused, but its specific activity was less than 30% of that of plasma acetoacetate. The lack of isotopic equilibration between plasma acetoacetate and  $\beta$ -hydroxybutyrate indicates that the ketone bodies do not exist as parts of a single, rapidly mixed pool under the circumstances of our experiments. Bates et al. (18) studied turnover of ketone bodies in fasting and diabetic rats by measuring specific activities of the ketone bodies at different times after a single injection of tracer amounts of  $\beta$ -hydroxybutyrate-<sup>14</sup>C. In their studies, the specific activity of acetoacetate averaged only 77% of that of  $\beta$ -hydroxybutyrate after injection of labeled  $\beta$ -hydroxybutyrate. They also observed that injected radioactive acetoacetate did not equilibrate rapidly between the two ketone body pools. Since equilibration is incomplete when tracer amounts of labeled ketone bodies are infused (18) (Table II), the turnover of plasma total ketone bodies cannot be determined accurately by the tracer technique. Bergman, Kon, and Katz (28) found in sheep infused continuously with large amounts of acetoacetate containing <sup>14</sup>C that the label equilibrated between plasma acetoacetate and  $\beta$ -hydroxybutyrate after 1 hr.

Bates et al. (18), assuming that the difference in specific activity between acetoacetate and  $\beta$ -hydroxybutyrate in their study was negligible, calculated the turnover of total ketone bodies after a single injection of labeled  $\beta$ -hydroxybutyrate (tracer amounts). The value they calculated for the turnover of total ketone bodies in rats fasted 2 days, 4.5  $\mu$ moles/100 g body weight per min, is similar to that calculated for  $\beta$ -hydroxybutyrate in the present study, 5.6  $\mu$ moles/100 g body weight per min (Table II). The slower turnover of acetoacetate, 2.5  $\mu$ moles/100 g body weight per min (Table II), may be related to the lower plasma concentration of acetoacetate since the fractional clearances of the two ketone bodies are similar (2).

It is generally accepted that during fasting ketone bodies are derived primarily from plasma FFA and that ketone bodies are readily utilized by many tissues (1, 3, 5, 6, 25, 28-30). The turnover rates for plasma FFA and  $\beta$ -hydroxybutyrate observed in rats in the present study, 3.2 and 5.6  $\mu$ moles/100 g body weight per min, respectively, suggest that at least 40% of the FFA mobilized during fasting may be metabolized through the plasma ketone body pool.

#### ACKNOWLEDGMENT

The authors are indebted to Miss Edna Jacobs for her excellent assistance in these studies.

<sup>1</sup> Clark, C. M., Jr., and R. O. Scow. Unpublished observations.

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