Effects of Fasting and Hypophysectomy on FFA Uptake and Ketone Body Production by the Isolated, Perfused Rat Liver

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SUMMARY

The effect of fasting overnight and hypophysectomy on free fatty acid (FFA) uptake and ketone body production by isolated, perfused rat livers was investigated. FFA uptake was directly related to FFA concentration over a wide range of concentrations (0.31 to 2.41 mM) in both fed and fasted rats. Whereas ketone body production increased with FFA uptake, it was greater at any given FFA concentration in livers from overnight fasted than in livers from fed donors. The relationship between FFA concentration and FFA uptake was unaltered by hypophysectomy. At low FFA concentrations livers from fed hypophysectomized rats demonstrated an increased rate of ketone body production as compared to those from fed normal rats. Livers from hypophysectomized rats fasted overnight produced ketone bodies at an increased rate as compared to livers from fed hypophysectomized rats, but the difference was significant only at high FFA concentrations. Diabetes *19*:924-29, December, 1970.

The physiologic alterations underlying the prevention of diabetic ketosis by hypophysectomy are poorly defined.[1-3] Hyperketonemia results from overproduction of ketone bodies[4-8] and is associated with a high concentration of free fatty acids (FFA) in the plasma.[9-10] Since FFA are taken up and oxidized by the liver in proportion to their concentration,[11-13] a reduction of FFA mobilization could reduce hepatic ketone body production. It has been suggested that hypophysectomy prevents ketosis in the diabetic animal by reducing FFA mobilization from adipose tissue.[14]

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The relationship between ketone body production and plasma FFA concentration can be influenced by hormonal and nutritional factors.[8,13,15-17] Insulin reduces ketone body production in the presence of a constant plasma FFA concentration in the fasting rat.[16] Glucagon increases ketone body production at low FFA concentrations in the isolated, perfused rat liver.[13] Ketone body production is higher in perfused livers from fasted rats than in livers from fed rats, at both low and high media FFA concentrations.[8] The effect of hypophysectomy on hepatic FFA uptake and ketone body production has not been previously investigated. The present experiments were designed to clarify the effects of fasting and hypophysectomy on FFA uptake and ketone body production by the isolated, perfused rat liver.

METHODS

Animals: Charles River strain rats, weighing 175-225 gm., were used throughout the study. They were housed in individual cages and fed Purina Laboratory chow. Hypophysectomized rats were also obtained from Charles River Breeding Laboratories. They were received one week after hypophysectomy and were kept at least one week before being used. All hypophysectomized rats were examined at necropsy to insure that hypophysectomy was complete. Fasting animals always had free access to water.

Perfusion: The livers were perfused by the technic of Mortimore[18] modified so that the perfusing medium did not recirculate. The perfusing medium was a suspension of red blood cells (20 ml./100 ml.) in 4 per cent albumin solution (Armour bovine plasma albumin powder, Fraction V, lot D27912) in modified Tyrode's solution.[19] The glucose concentration was 1 mg./ml. Red blood cells prepared from defibrinated rat blood[20]

were used in earlier experiments and bovine red blood cells, prepared as below, were used in later experiments. The change in cells did not alter the results and the data have been combined. The bovine cells were separated from plasma by centrifuging citrated (A.C.D. solution, U.S.P., 45 ml./liter of whole blood) blood in a refrigerated centrifuge at 2,000 PRM (2,500 X G) for fifteen minutes. The cells were then washed twice with a volume of 0.9 per cent NaCl solution equal to the plasma removed and recentrifuged. The cells were separated from plasma and washed on the day of use; the blood was obtained fresh weekly. FFA added to the medium were prepared by saponifying rat adipose tissue and extracting the FFA with hexane. The hexane was then evaporated and the FFA were dissolved in 0.154 M $NaHCO_3$ at a concentration of 10 μEq./ml. Appropriate amounts of the latter were added to 4 per cent albumin solution, *p*H was adjusted to 7.4 with NaOH, and the mixture was filtered through a 0.2 micron milipore filter. Red blood cells were then added. The livers were perfused at a rate of 5 ml. per minute per liver; the livers weighed between 6 and 8 gm.

Analyses: Plasma FFA were measured by a modifica- of the Dole technic[19] and blood total ketone bodies (TKB) by the method of Chernick.[21] Arterial blood samples were obtained from the reservoir throughout the perfusion. Since the arterial FFA and TKB concentrations did not change during the perfusion, the values were averaged. (The arterial TKB concentrations, of course, were negligible.) Venous blood was collected in ice cold tubes over the time periods indicated and analyzed. Arterial-venous differences and blood flow rates were determined and calculations were made as follows:

$$\text{FFA uptake } (\mu\text{mole} \times \text{gm.}^{-1} \times \text{hour}^{-1}) = \frac{[\text{Arterial plasma FFA } (\mu\text{mole} \times \text{ml.}^{-1}) - \text{Venous plasma FFA } (\mu\text{mole} \times \text{ml.}^{-1}] \times \text{flow (ml.} \times \text{hour}^{-1}) \times [1 - \text{Hct}]}{\text{liver wt. (gm.)}}$$

$$\text{TKB production } (\mu\text{mole} \times \text{gm.}^{-1} \times \text{hour}^{-1}) = \frac{[\text{Venous blood TKB } (\mu\text{mole} \times \text{ml.}^{-1}) - \text{Arterial blood TKB } (\mu\text{mole} \times \text{ml.}^{-1})] \times \text{flow (ml.} \times \text{hour}^{-1})}{\text{liver wt. (gm.)}}$$

The statistical significance of differences was determined by Student's "*t*" test.

RESULTS

The effect of overnight fasting on FFA uptake and ketone body production

Rates of FFA uptake and TKB production by perfused livers from fed rats and from rats fasted overnight are shown in figure 1. The livers were perfused for one hour with medium containing 0.78 and 0.93 mM FFA, respectively. The uptake of FFA was constant and similar for the two groups, 12.1 and 12.6 μmoles x gm.$^{-1}$ x hour^{-1}, respectively. TKB production, however, was higher in the livers from fasted rats (38.4 μmoles x gm.$^{-1}$ x hour^{-1} during the first and 31.7 μmoles x gm.$^{-1}$ x hour^{-1} during the second 30 minutes) than in the livers from fed rats (15.5 μmoles TKB x gm.$^{-1}$ x hour^{-1}) ($p < .01$ and $p < .02$) (figure 1).

The effect of alterations in FFA concentration on FFA uptake and ketone body production

The effect of increasing plasma FFA concentration on FFA uptake and TKB production in livers from fed and fasted rats is summarized in figure 2. Plasma concentration was increased from 0.31 to 1.11 mM for the livers from fed and from 0.40 to 1.41 mM for the livers from fasted donors. In both groups of livers FFA uptake increased significantly during the first five-minute collection period after the FFA concentration was increased ($p < .001$) and remained constant throughout the remainder of the perfusion. There were no significant differences in FFA uptake between livers from fed and fasted rats at either FFA concentration.

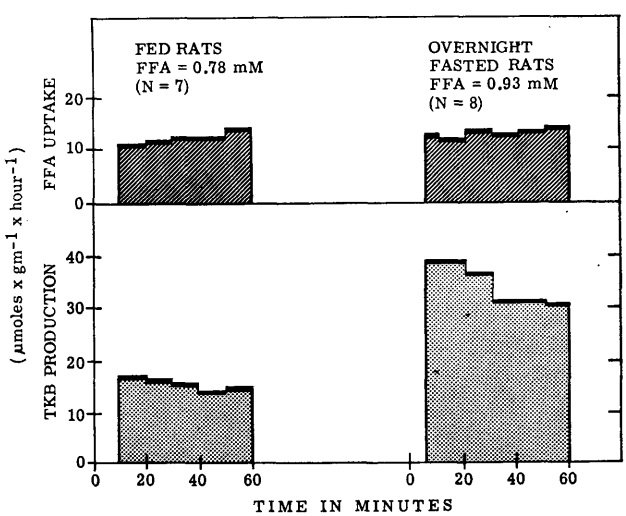

FIG. 1. FFA uptake and total ketone body (TKB) production in perfused livers from fed and overnight fasted rats. Mean values are plotted.

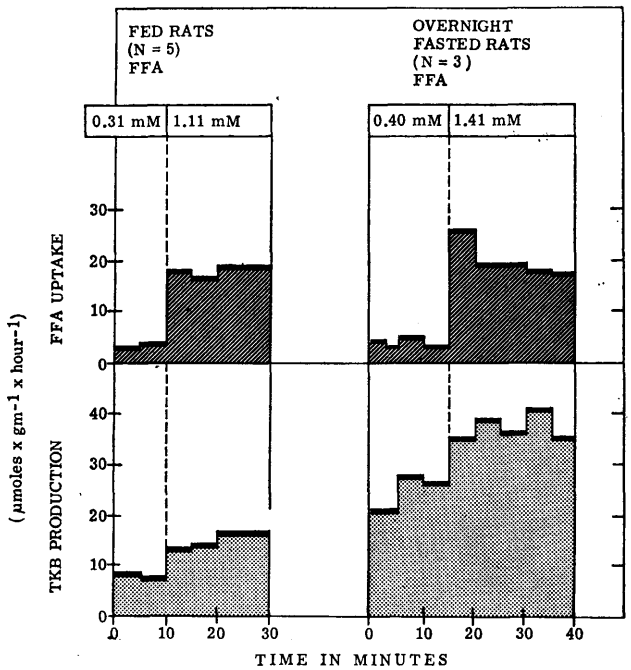

FIG. 2. (left) The effect of increasing FFA concentration on FFA uptake and TKB production in perfused livers from fed and overnight fasted rats. Mean values are plotted.

TKB production also increased during the first five-minute interval after elevating the plasma FFA concentration, but the changes did not become significant until the second interval. The mean rate of TKB production in livers from fed rats was 7.0 μmoles x gm.$^{-1}$ x hour^{-1} at low FFA concentrations and 14.8 μmoles x gm.$^{-1}$ x hour^{-1} at high FFA concentrations ($p < .001$). The values for livers from fasted rats were 24.2 and 37.3, respectively ($p < .001$).

The relationship between FFA concentration and FFA uptake

The relationship between FFA concentration and FFA uptake in livers from fed donors is summarized in the center panel of figure 3. There is a high degree of correlation ($r = 0.93$, $p < .001$) between FFA concentration and uptake. Similar data for livers from fasted animals are summarized in the left hand panel of figure

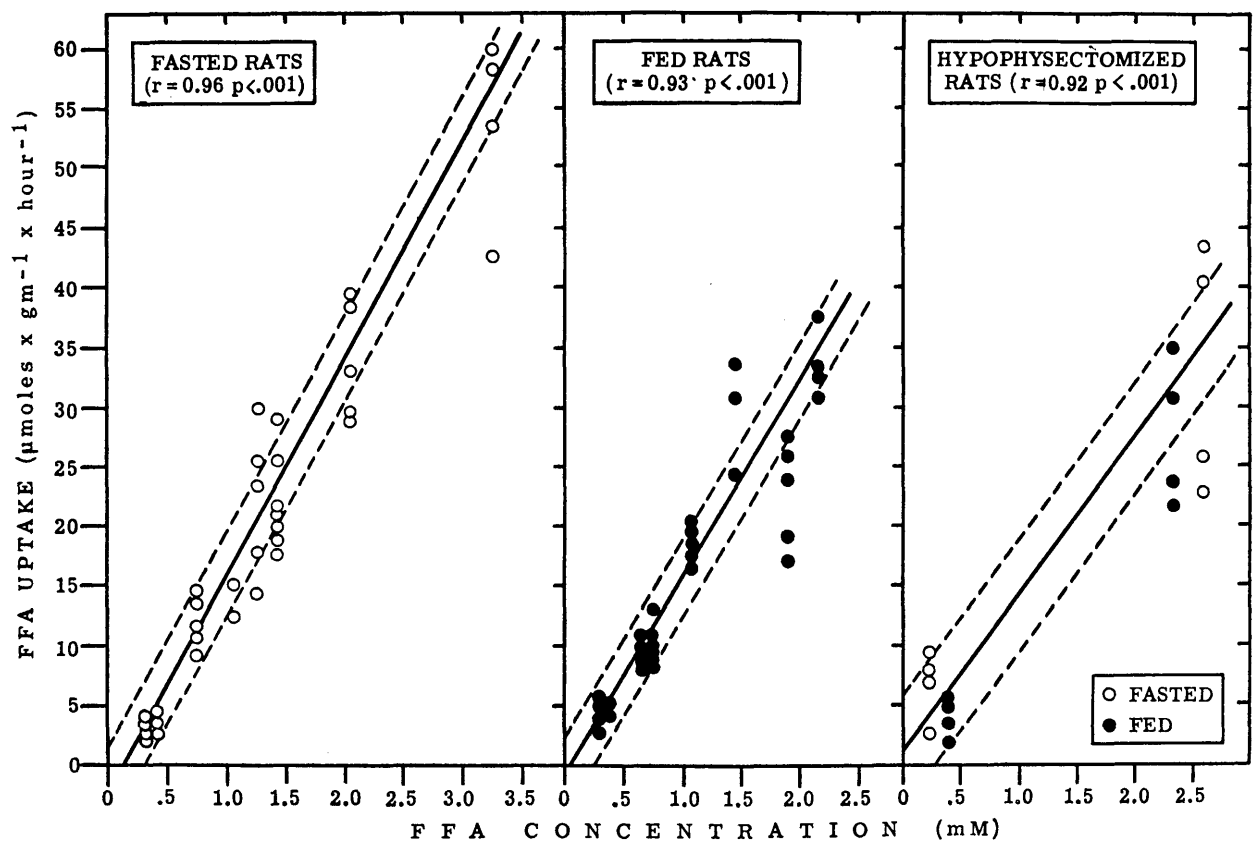

FIG. 3. The effect of FFA concentration on FFA uptake in perfused livers from fed, fasted and hypophysectomized rats. Each dot represents the average FFA uptake for one liver over a thirty-minute perfusion. Regression lines and their standard deviation are plotted.

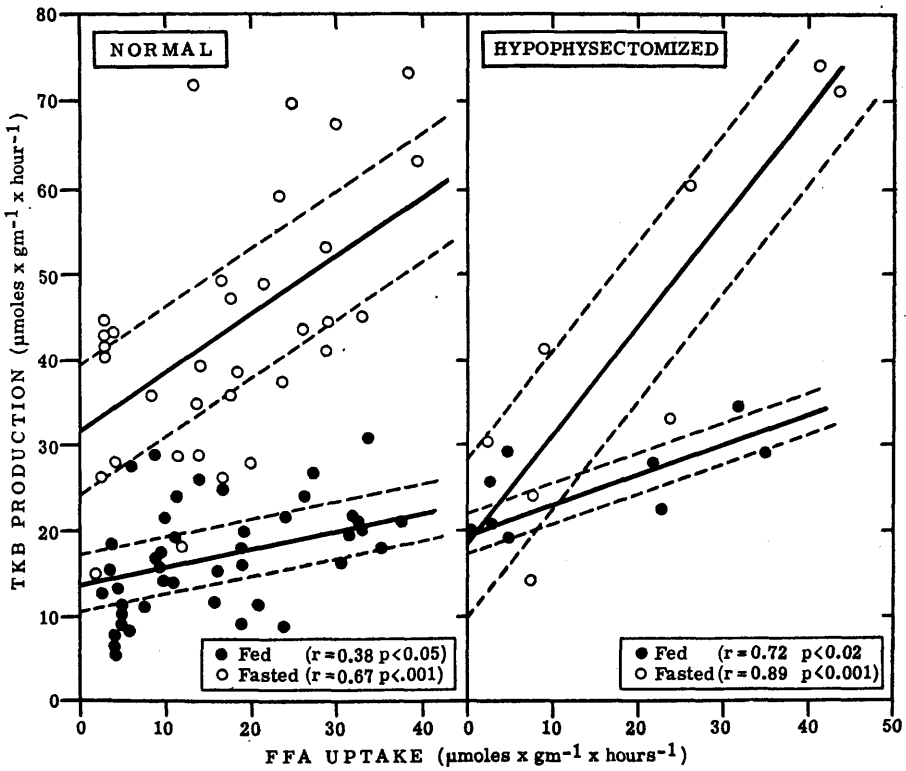

FIG. 4.

The effect of FFA uptake on TKB in perfused livers from fed and fasted normal and hypophysectomized rats.

Hypophysectomies were performed at least two weeks prior to the perfusions. The fasted rats were deprived of food overnight. Each dot represents the average total ketone body production for a thirty-minute perfusion. The regression lines and their standard deviations are plotted.

3. The regression line appears identical to that of the livers from fed animals ($r = 0.96$, $p < .001$).

The relationship between FFA uptake and ketone body production

The relationship between FFA uptake and TKB production in livers from fed and fasted normal rats is summarized in the left hand panel of figure 4. There was a significant correlation between FFA uptake and TKB production in both groups. TKB production, however, was significantly greater in livers from fasted than from fed rats.

The effect of hypophysectomy on FFA uptake and TKB production

Livers from fed and overnight-fasted hypophysectomized rats were perfused at two FFA concentrations, 0.30-0.40 and 2.30-2.50 mM. FFA uptake was highly correlated with FFA concentration ($r = 0.92$, $p < .001$) and was similar to that seen in normal rats (right hand panel of figure 3).

At low FFA concentrations, mean TKB production by livers from fed hypophysectomized rats was 21.7 μmoles x gm.$^{-1}$ x hour^{-1}. This was significantly greater than that observed in livers from fed normal rats, 12.9 μmoles x gm.$^{-1}$ x hour^{-1} ($p < 0.05$) (figure 4). At higher FFA concentrations this difference was not observed. Fasting overnight increased TKB production in livers from hypophysectomized rats from a mean of 21.7 to 28.2 μmoles x gm.$^{-1}$ x hours^{-1} at low FFA concentrations, but this increase was not significant ($p < 0.1$) (figure 4). TKB production by livers perfused at high FFA concentrations, however, was significantly increased, from 28.8 to 60.2 μ moles x gm.$^{-1}$ x hour^{-1}, by the overnight fast ($p < 0.01$). There was no significant difference at either FFA concentration in TKB production between livers from hypophysectomized rats fasted overnight and livers from normal rats fasted overnight (figure 4).

DISCUSSION

The data presented confirm earlier observations that uptake of FFA and ketone body formation by the perfused liver are both linearly related to the plasma concentration of FFA.[11-13] Although fasting did not affect the uptake of FFA (figure 3),[22] it increased the amount of ketone bodies produced by the liver (figure 4). This effect on ketogenesis is undoubtedly mediated through changes in the metabolic fate of FFA in the liver,[23,24] with proportionally more FFA being oxidized to ketone bodies.[25] These changes may be secondary to decreased availability of glucose during fasting.[24,25] Although fasting increases the activity of carnitine acyltransferase in the liver,[26,27] studies with isolated mitochondria suggest

that this enzyme is not rate limiting in the oxidation of fatty acids.[28] Assays of other enzymes involved in the formation of ketone bodies indicate that their activities are not increased by fasting.[17,29] Further studies are needed to determine the changes in liver that enhance ketone body formation during fasting.

Development of ketosis in pancreatectomized[1-3] and pregnant rats[30] is markedly decreased by hypophysectomy. The present study shows that hypophysectomy does not affect the uptake of FFA (figure 3). The formation of ketone bodies in the isolated, perfused liver from fed rats was increased by hypophysectomy at low FFA concentrations (figure 4). This may be explained on the basis of the decreased availability of glucose seen in hypophysectomy,[31] allowing more FFA to be oxidized as in the fasted animals.[24,25] The increased ketone body production by livers from hypophysectomized rats produced by increasing FFA concentration and by fasting suggests that the pituitary hormones do not have a direct effect on these aspects of lipid metabolism in the liver (figure 4). Other studies have shown that glucagon[13] and insulin[16] affect ketone body production independent of the effects they have on plasma FFA concentration.[9] It is well known that hypophysectomy decreases FFA mobilization in fasting[32] and diabetic animals.[14] The results of the present study support the hypothesis[14] that hypophysectomy prevents ketosis in the diabetic rat by decreasing FFA release by adipose tissue.

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Sugar and Coronary Heart Disease

(*Continued from page* 923)

en were resurveyed two years later and the simple correlation coefficient between the two estimates was 0.8 ($p < 0.001$).

The male community survey included 344 men (79 per cent of an age and occupation stratified sample who had been examined in 1958). Electrocardiograms as well as the same questions used in the female sample were collected. Neither on the basis of angina pectoris nor on the basis of the electrocardiographic patterns was there any demonstrable relationship between consumption of sugar and CHD. Here too, sugar consumption per day was greater among pack-a-day smokers (103 gm.) than nonsmokers (83 gm.). Thus both of these studies suggested a relationship between the use of sugar and of smoking habits, a confounding factor in relating sugar to CHD.

A companion article by A. E. Bennett, R. Doll, and R. W. Howell (Lancet 1:1011, 1970) lends further support to this observation. This report is actually of three separate surveys. Among male hospitalized patients aged thirty-five through sixty-four, mean daily sugar use in hot beverages ranged from 34.6 gm. among nonsmokers to 53.4 among smokers of fifteen or more cigarettes per day. Data on 2,483 males forty through fifty-four years of age (representing a 90 per cent response of a random sample of industrial workers) related smoking habits to a questionnaire-derived estimate of sugar consumption. Total daily sugar use was higher among cigarette smokers than nonsmokers, the difference being accounted for by sugar consumption in hot drinks. Lastly, a random sample of 453 males twenty-six through sixty-five years of age residing in a defined area also yielded data showing that heavy cigarette smokers used more hot beverages (8.1 cups per day compared with 5.2 in light smokers and 4.8 in nonsmokers) as well as more sugar per cup (9.1 gm. among heavy smokers, 7.8 gm. among light smokers). Considering all of these studies together, there was noted a significant association ($p < 0.05$) between cigarette smoking and both hot beverage use and sugar consumption.

Various speculations about this association were raised by Bennett et al. Are both smoking and tea or coffee drinking related to some underlying psychological need or are they merely socializing habits shared by chance? Could oral-pharyngeal dryness in smokers lead to a greater use of beverages? Does the greater use of sugar per cup of hot beverage among smokers suggest an altered taste threshold for sweetness. Whatever the mechanisms involved, it now appears that the apparent association between CHD and sugar is neither as straightforward nor as clear as originally hypothesized.

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