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Correlation Between Concentrations of Circulating Free Fatty Acids and Ketone Bodies.* (30768)

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In fasting and diabetic animals there is an elevated level of both free fatty acids (FFA) and ketone bodies in the blood(1). The major source of plasma FFA is adipose tissue (2) whereas the blood ketone bodies originate in the liver and are largely derived from the oxidation of fatty acids(3). In fasting and diabetic states many other metabolic alterations occur in addition to the increase in plasma FFA level. It therefore remains obscure whether the plasma FFA elevation *per se* is the cause of ketosis or whether this condition is a combined result of several concomitant metabolic alterations. To clarify this point the plasma FFA level was elevated directly in the present investigation by the administration of heparin which does not present other metabolic complications.

The plasma level of FFA was increased in normal, well-nourished rats by administration of corn oil and subsequent heparin injection. By this treatment plasma FFA are produced by the hydrolysis of chylomicron triglycerides catalyzed by lipoprotein lipase (4). The effects of this treatment on the concentration of both blood ketone bodies and plasma FFA were determined.

Methods. Preliminary experiments were conducted to determine the duration of the elevated plasma FFA post-heparin. Male Holtzman adult rats, 250-350 g, were fed Purina laboratory chow and water *ad libitum*. Without prior fasting, corn oil, 0.8 ml/100 g body weight, was fed by stomach tube to induce chylomicronemia. Two hours after corn oil administration a blood sample was removed by heart puncture during ether anesthesia. By leaving the hypodermic needle in the heart after the syringe containing the blood was removed, 1 mg of heparin/kg body weight was immediately injected. The hepa-

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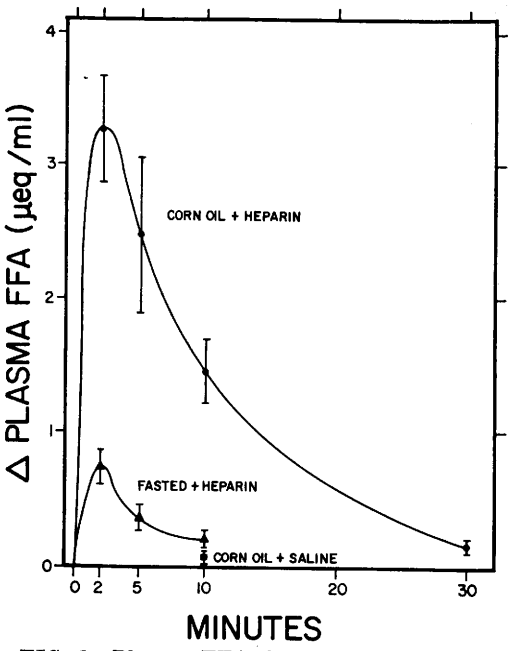

FIG. 1. Plasma FFA level after intravenous heparin, 1 mg/kg body weight, in fasted and corn oil-fed rats. Each point represents 4 animals except at 10 min which represents 11 animals in each group. Ranges shown are standard errors of mean.

rin solution contained 1 mg heparin/ml of 0.9% NaCl. Another blood sample was removed from each rat either 2, 5, 10 or 30 minutes later. Similarly, blood from rats fasted 12 hours was removed just prior to heparin injection and either 2, 5 or 10 minutes later. Blood samples were collected in tubes containing 1 drop of heparin solution per ml of blood. To prevent lipolysis *in vitro,* blood samples were kept ice-cold from the time of removal until extraction for FFA analysis by the procedure of Dole and Meinertz(5), with Nile blue as indicator.

Results and discussion. As shown in Fig. 1, the plasma FFA level after heparin treatment increased markedly in the corn oil-fed rats and then declined to the pre-heparin range. The major component of this decline is probably due to depletion of plasma chylomicrons, although the concurrent removal of lipoprotein lipase activity from the blood may be contributory. The mild heparin-induced elevation of plasma FFA in the fasting animals is probably a result of the action of released lipoprotein lipase on plasma lipoproteins(6). From the pattern of the post-heparin elevation of plasma FFA, it was decided that blood ketone bodies and FFA would be measured at zero time and 10 minutes after the injection of heparin in corn oil-fed rats (Group III). Control oil-fed rats (Group II) were treated similarly except that 0.9% saline was injected intracardially instead of the heparin solution. For comparison, rats were also fasted for 12 hours, blood was removed, heparin injected, and more blood was removed 10 minutes later as described above (Group I). Between the zero time and 10 minutes post-injection blood samples the rats were not under ether anesthesia. Ketone bodies were measured in whole blood by the method of Lyon and Bloom(7), as modified by Ontko and Jackson(8), and reported as acetone. In this analysis the concentration of total ketone bodies in rat plasma and whole blood was found to be essentially the same.

The differences between the ketone and FFA concentrations at zero time and 10 minutes after heparin or saline injection are shown in Table I. At zero time the average levels of plasma FFA in the rats in Group I, Group II and Group III were 0.460, 0.431 and 0.538 μeq/ml respectively. The corresponding concentrations of blood ketone bodies at zero time were 1.67, 1.51 and 1.85 mg% expressed as acetone. In the animals fed corn oil, the plasma FFA concentration 10 minutes post-heparin was significantly elevated relative to both the corn oil-fed saline-injected rats and fasted heparin-treated animals. In agreement with Fig. 1, the plasma FFA level in the fasted rats was elevated 10 minutes post-heparin but was unchanged in corn oil-fed rats treated with saline. The blood level of total ketone bodies paralleled the rise in plasma FFA. In the corn oil-fed rats the blood ketone body concentration was markedly increased 10 minutes after heparin injection but was unaltered by saline treatment. An increase in blood ketone body level in the fasted rats post-heparin accompanied the moderate elevation in plasma FFA in these animals.

Fatty acids in the liver participate in many processes in addition to oxidation. Altera-

TABLE I. Heparin-Induced Increase in FFA and Ketone Bodies.
Values shown are the average differences between FFA and ketone body concentrations at zero time and 10 min after indicated treatment.

Treatment (7 rats/group)	Change in plasma FFA μeq/ml $\pm$ st error of mean	Change in blood ketone bodies mg % $\pm$ st error of mean
I Fasted, heparin-injected	+ .186 $\pm$ .049	+ .73 $\pm$ .27
II Corn oil-fed, saline-injected	+ .071 $\pm$ .038	+ .25 $\pm$ .19
III Corn oil-fed, heparin-injected	+1.208 $\pm$ .138	+1.59 $\pm$ .23

FFA:		Ketone bodies:	
I *vs* III	$P < .001$	I *vs* III	$P < .05$
II *vs* III	$P < .001$	II *vs* III	$P < .005$
I >0*	$P < .005$	I >0*	$P < .025$
II >0*	$P < .1$	II >0*	$P < .25$

* Significance level of paired differences between values at zero time and 10 min after treatment.

tions in chain length and degree of saturation, glyceride and phosphatide formation, complete oxidation to CO_2: all are possible routes in addition to partial oxidation to generate ketone bodies. A variety of control mechanisms may regulate the rates of these processes. Therefore an increased influx of FFA into the liver does not necessarily lead to an increase in production of all possible products. Palmitic acid at low concentrations undergoes oxidation preferentially to CO_2 in liver homogenates whereas at higher concentrations the fatty acid is predominantly oxidized to ketone bodies in this *in vitro* system(9). The results presented herein suggest that also *in vivo* elevation of the plasma FFA *per se* can cause a significant elevation in hepatic ketogenesis. Although other factors not directly related to the elevated influx of FFA into the liver may serve to augment ketogenesis in this organ in states of ketosis, at least part of the ketosis observed in these conditions can be accounted for on the basis of elevated plasma FFA alone.

Summary. After injection of heparin into corn oil-fed rats the level of plasma FFA was determined as a function of time. In the absence of metabolic alterations induced by fasting or by hormone administration or deletion, an elevation in plasma FFA was accompanied by a concomitant elevation in the concentration of blood ketone bodies. Control corn oil-fed rats injected with saline showed no such response. The results indicate that an increased level of plasma FFA *per se* can accelerate hepatic ketogenesis.

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