PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, 147 578-580 (1974)

Studies on the Effects of Tricaprylin on Gluconeogenesis and Ketogenesis in Isolated Perfused Liver[1] (38391)

W. R. INGEBRETSEN, JR.,[2] AND S. R. WAGLE

Department of Pharmacology, Indiana University School of Medicine, Indianapolis, Indiana 46202

In recent years, interactions between carbohydrates and fatty acid metabolism has been extensively investigated. In the liver, fatty acids can inhibit glycolysis and stimulate gluconeogenesis but more importantly, carbohydrates have an inhibiting effect on fatty acid oxidation and on ketogenesis. The antiketogenic effects of carbohydrates has been shown by *in vitro* studies using ^{14}C fatty acids to be due to a sparing action on fatty acid oxidation (1–3). In the present studies, we wish to report the effects of tricaprylin on gluconeogenesis and ketogenesis in the presence of various carbohydrate precursors using perfused liver.

Materials and Methods. Animals. Fasted (18–24 hr) male Cox rats weighing 180–200 g were used in all of these studies. Rats were fed Purina Laboratory Chow and water and maintained under standard laboratory conditions until withdrawn from food.

Liver perfusion. Normal fasted rats were anesthetized with Na pentobarbital. The liver was removed and perfused for 30 min with 100 ml of Umbreit–Ringer bicarbonate buffer containing 1.5 g of albumin (Sigma, Fraction V). At the end of 30 min of preperfusion, various glycogenic substrates were added and perfusion continued for an additional 30 min. At this time period fatty acid tricaprylin was added; perfusion was continued for an additional 30 min. A sample of the perfusate was taken at 30 min intervals to measure glucose and ketone body production. Glucose was measured by the glucose oxidase method (4) and ketone bodies were measured by the phlorometric method (5).

Results and Discussion. The effect of alanine, pyruvate, galactose, lactate and tricaprylin on ketogenesis is summarized in Table I. These results indicate that the addition of 10 m*M* galactose, lactate, pyruvate or alanine to the perfusion medium suppress the formation of ketone bodies by 50–75%. Since these agents suppressed endogenous ketogenesis, further experiments were undertaken to determine if tricaprylin could stimulate ketogenesis and if this stimulation could be inhibited by these substrates. It can be seen from Table I that the addition of 1 m*M* tricaprylin in the absence of carbohydrate caused a twofold increase in ketogenesis. Alanine, pyruvate or galactose had no effect on the increase in ketogenesis in the presence of tricaprylin. Only lactate suppressed the increase in ketogenesis in the presence of tricaprylin. The antiketogenic effect of lactate in the presence of tricaprylin is of interest since the oxidation of the fatty acid moiety of tricaprylin (octanoate) has been shown not to be effected by carbohydrates (6). Hence experiments were undertaken to determine if lactate would suppress ketogenesis from the octanoate in the present system. The results of these studies are presented in Table II. The addition of 1, 2, or 3 m*M* octanoate causes up to a threefold increase in ketogenesis. Lactate addition had no effect on the increase in ketone body formation in the presence of octanoate.

As it is well known that fatty acid affects glucose production, experiments were also undertaken to study gluconeogenesis under the above experimental conditions. The results of those studies are given in Table III. It can be seen from this table that tricaprylin stimulated glucose production in the presence of 10 m*M* lactate, alanine or galactose. The stimulation ob-

[1] Supported by a grant from Eli Lilly and Company.

[2] Present Address: Department of Pharmacology and Medicine, University of California, San Diego, La Jolla, CA.

Printed in U.S.A.

TABLE I. Effect of Various Substrates on Ketogenesis in the Presence of Tricaprylin in Isolated Perfused Liver.* (μmoles ketones/g/hr)

10 m*M* Substrate	No tricaprylin	1 m*M* Tricaprylin	Increase in Ketogenesis in presence of tricaprylin
No substrate	39.0 ± 2.8 (31)	77.2 ± 6.5 (16)	+38.2 ± 3.4 (16)
Alanine	12.4 ± 1.9 (15)	42.6 ± 6.2 (9)	+30.2 ± 4.4 (9)
Pyruvate	9.0 ± 3.7 (10)	49.7 ± 5.6 (9)	+40.7 ± 4.5 (9)
Galactose	19.1 ± 2.2 (15)	56.7 ± 6.5 (8)	+37.6 ± 4.4 (8)
Lactate	9.8 ± 1.7 (17)	24.3 ± 4.6 (4)	+14.5 ± 2.7 (4)

*Livers were perfused as described in Materials and Methods. Values are mean ± SEM of (*N*) observations.

tained with pyruvate was not significant. Tricaprylin is composed of three fatty acids esterified to glycerol. It is possible that either the glycerol or the octanoate moiety of tricaprylin could stimulate gluconeogenesis. Tricaprylin had no effect on glucose production (Table III) in the absence of another added gluconeogenic substrate would suggest that glycerol moiety was not responsible for the increase in glucose production. Thus, the increase in glucose production may be due to the octanoate moiety of tricaprylin. This was further tested by using various concentrations of octanoate on glucose production. It can be seen from Table III that the addition of octanoate (1 or 3 m*M*) to the perfusion medium did stimulate glucose production from lactate. Octanoate alone had no effect on glucose production. Octanoate and tricaprylin at 1 m*M* concentration were equally effective in stimulating glucose production from lactate. However the rate of ketogenesis under these conditions was remarkably dissimilar. Octanoate-stimulated ketogenesis was twice that observed with tricaprylin at the same concentration. These data are in agreement with previously reported observations that there is no correlation between rates of gluconeogenesis and ketogenesis (7–9).

The finding that triglycerides or free fatty acids stimulate glucose production is in agreement with recent reports that fatty acid oxidation is needed for high rates of glucose production. Frohlich and Wieland (7) demonstrated that rates of glucose production in the presence of 10 m*M* lactate were reduced by glycodiazine, an agent which blocks triglyceride hydrolysis but not *beta* oxidation. Furthermore, the stimulation of glu-

TABLE II. Effect of Antiketogenic Substrates on Ketogenesis in the Presence of Octanoate in Isolated Perfused Liver.*

(μmoles ketones/g/hr)

	No lactate		10 m*M* Lactate	
Octanoic acid	Ketone body formation	Net increase	Ketone body formation	Net increases
None (Endogenous)	28.6 ± 2.2 (17)	—	8.5 ± 1.6 (17)	—
1 m*M*	64.0 ± 14.4 (6)	+35.4 ± 7.7 (6)	48.6 ± 8.1 (6)	+40.1 ± 6.5 (6)
2 m*M*	113.3 ± 13.5 (5)	+84.7 ± 8.6 (5)	96.7 ± 10.5 (5)	+88.2 ± 9.2 (5)
3 m*M*	119.1 ± 11.2 (6)	+90.5 ± 9.1 (6)	86.4 ± 3.4 (6)	+77.9 ± 3.1 (6)

*Livers were perfused as described in Materials and Methods. Values are the mean ± SEM of (*N*) observations.

TABLE III. Effects of Tricaprylin and Octanoate on Glucose Production in Isolated Perfused Liver.* (μmoles glucose/g/hr)

10 m*M* Substrate	No lipid addition	Tricaprylin 1 m*M*	Octanoate 1 m*M*	Octanoate 3 m*M*
No substrate	7.2 ± 1.3 (14)	7.9 ± 1.4 (15)	12.0 ± 2.7 (4)	11.9 ± 1.8 (6)
Lactate	29.2 ± 5.7 (4)	53.5 ± 7.0 (4)	55.0 ± 12.7 (6)	73.3 ± 3.2 (6)
Alanine	20.7 ± 1.1 (5)	39.0 ± 3.8 (9)	—	—
Pyruvate	32.8 ± 6.6 (5)	42.5 ± 6.8 (5)	—	—
Galactose	19.3 ± 2.3 (10)	29.4 ± 4.0 (10)	—	—

*Livers were perfused as described in Materials and Methods. Values are mean ± SEM of (*N*) observations.

cose production by glucagon in the presence of lactate was completely reversed by glycodiazine. The stimulatory effect of glucagon in the presence of glycodiazine was restored by oleic acid. McGarry *et al.* (10) demonstrated the decarnoylcarnitine reduced high rates of glucose production (10 m*M* lactate) but had no effect on low rates of glucose production (2 m*M* lactate). Fatty acid oxidation is necessary for maintaining high gluconeogenic rates but is not essential for low rates of glucose production. Therefore, fatty acid oxidation may regulate gluconeogenesis under conditions of increased availability such as hormonal stimulation, starvation or diabetes.

Summary. The effects of gluconeogenic substrates on ketogenesis and the effects of tricaprylin on gluconeogenesis were studied using perfused rat liver. Galactose, lactate, pyruvate or alanine (10 m*M*) suppressed endogenous ketogenesis. Tricaprylin (1 m*M*) caused a twofold increase in ketogenesis which was suppressed by lactate but not by galactose, alanine and pyruvate. Lactate did not suppress the increase in the presence of octanoate. This suggests that the inhibition of ketogenesis by lactate may involve the suppression of triglyceride hydrolysis. Tricaprylin stimulated glucose production in the presence of lactate, galactose, or alanine. The increase in glucose production appeared to be due to the octanoate moiety of tricaprylin.

1. Weinhouse, S., Millington, R. A., and Friedman, B., J. Biol. Chem. **181,** 489 (1949).

2. Lossaw, W. J., and Chaikoff, I. L., Arch. Biochem. Biophys. **57,** 23 (1955).

3. Allen, A., Griedman, B., and Weinhouse, S. J. Biol. Chem. **212,** 921 (1955).

4. Hugget, A., and Nixon, S. N., Lancet **2,** 268 (1957).

5. Young, R., and Renold, A. E., Clin. Chem. Acta **13,** 791 (1966).

6. McGarry, J. B., and Foster, D. W., J. Biol. Chem. **246,** 1149 (1971).

7. Frohlich, J. and Wieland, O. *in* "Regulation of Gluconeogenesis" (H. D. Soliny, and B. Williams, eds.), p. 179. Academic Press, New York (1971).

8. Exton, J. H., Corbin, J. G., and Parks, C. R., J. Biol. Chem. **244,** 4095 (1969).

9. Ross, B. D., Hems, S., and Krebs, H. A., Biochem. J. **102,** 942 (1967).

10. McGarry, J. D., Meier, J. M., and Foster, D. W., J. Biol. Chem. **248,** 270 (1973).

Received May 22, 1974. P.S.E.B.M., 1974, Vol. 147.