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# The effect of insulin and alloxan diabetes on hepatic transport of triglycerides and fatty acids*

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THE fatty liver and hyperlipemia associated with experimental and clinical diabetes has been well documented.[1] The etiology of these conditions, however, is poorly understood. In order to determine whether the fatty liver of diabetes was causally related to some defect in hepatic transport of triglyceride (TG), we investigated the effect of insulin and alloxan diabetes on the uptake of nonesterified fatty acids (NEFA) and on the release of TG by the isolated, perfused rat liver. It is clear from our data that insulin deficiency produced a marked inhibition of release of hepatic TG and that this inhibition may, in part, be responsible for the fatty liver of diabetes. Furthermore, in the presence of elevated concentrations of NEFA in the perfusate, the livers from alloxan-diabetic rats released net amounts of TG into the medium; this rate of TG release was considerably less than that by livers from normal animals given equivalent amounts of NEFA, but was of the same order of magnitude as the TG release by livers from normal animals exposed to low concentrations of NEFA *in vitro*.[2, 3] It is proposed, therefore, that the fatty liver and hyperlipemia of alloxan diabetes may result, in part from an inhibition of release of TG by the liver in the presence of increased levels of NEFA in the blood.

## MATERIALS AND METHODS

Livers, isolated from normal and diabetic rats, were perfused *in vitro*. The perfusion procedure[2, 3] and apparatus[4] have been described previously. Male rats (Holtzman Co., Madison, Wis.) weighing 250–350 g were used as the liver and blood donors. Blood was obtained by aortic puncture from normal male rats under light ether anesthesia. Blood obtained from normal animals was used to perfuse livers removed from either normal or diabetic rats. All animals were allowed free access to food (Purina lab chow) and tap water. The rats were made diabetic 48 hr prior to use by the i.v. injection of 60·0 mg alloxan monohydrate/kg. The rats were given 5–10 ml of 0·9% NaCl by i.p. injection immediately after the alloxan administration. The rats were not considered to be diabetic unless the blood sugar was in excess of 400 mg/100 ml.

The perfusion medium consisted of defibrinated blood diluted to three times its original volume with Krebs-Henseleit bicarbonate buffer, pH 7·4.[5] Twenty mg palmitic acid, as the palmitate–serum complex,[3] was added to the medium 20 min after insertion of the liver into the perfusion system. The final fluid volume before sampling was 130 ml. Three min after palmitate addition to the medium samples of perfusate were taken for "zero time" measurements. The perfusate was lightly centrifuged to sediment the erythrocytes; 2·0 ml of the supernatant was extracted in a 50-ml volume with chloroform : methanol (2 : 1, v/v), filtered, and aliquots of the filtrate washed with 0·5 volume of 0·02% $MgCl_2$. The $CHCl_3$ layer was evaporated to dryness *in vacuo*, and the residue was redissolved in a minimal volume of $CHCl_3$ and placed on a 2·0 g silicic acid column. Triglycerides present in chloroform eluates of the column were measured by the procedure of Van Handel and Zilversmit;[6] nonesterified fatty acids were estimated by the Duncombe method.[7] Blood glucose was determined according to the procedure of Nelson.[8]

## RESULTS

The rate of disappearance of palmitate from the perfusion medium is depicted in Fig. 1. It can be seen that palmitate disappears more rapidly from the medium when livers from alloxan-diabetic rats are perfused than when livers from normal animals are employed. The rate of disappearance of palmitate appears to be proportional to concentration in the medium. If one considers liver mass in calculation of rate of NEFA uptake, the rapid uptake of NEFA by livers from alloxan-diabetic animals is even more apparent (Table 1). Administration of insulin to the diabetic animals restored rates of NEFA uptake by the livers to control levels.

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The release of triglyceride appears to be severely inhibited in livers from alloxan-diabetic rats (Table 1). Under conditions of moderate fatty acid concentration in the medium (0·6 μmole/ml) there was actually a net uptake of TG from the medium by livers from diabetic animals. This is analagous to the inhibition of TG output that was observed in livers from 48-hr fasted rats.[2]

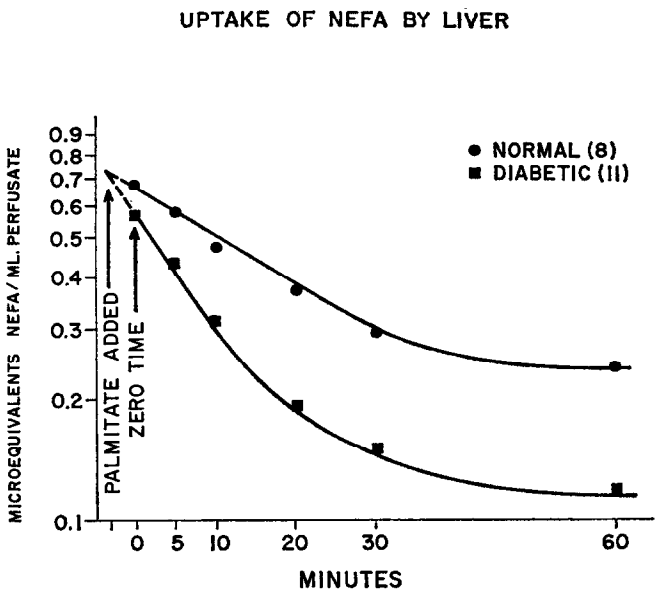


FIG. 1. The values for the normal control are significantly different from corresponding values for the diabetic, with $P < 0{\cdot}01$ for all points except at the 5-min period, at which time $P < 0{\cdot}025$; at zero time, P is not significant.

When larger amounts of palmitate were added to the perfusate, the inhibition of TG release by livers from diabetic rats was still evident (F, G; Table 1). Under these conditions, however, the livers from both normal and diabetic animals released TG into the medium. In order to rule out fasting as a responsible factor in the restriction of TG output by the livers from diabetic animals, experiments were carried out in which a purified diet was force fed (25 g/rat/day in three divided feedings for a period of 2 days) by stomach tube to normal and diabetic rats. The results of these preliminary experiments are in accord with data obtained from experiments using livers of animals allowed to feed *ad libitum*. Triglyceride release by the livers from alloxan-diabetic rats was partially reversed by the addition of crystalline zinc insulin to the medium. Two of the four livers in this latter group (E) had apparently normal TG output, whereas two were indistinguishable from untreated diabetics.

## DISCUSSION

A major pathway of nonesterified fatty acid metabolism in the liver is esterification to form triglycerides. The newly formed TG is rapidly transported into the serum in the very low-density lipoprotein ($d < 1{\cdot}006$). The transport and metabolism of NEFA and TG by the liver appear to be sensitive to changes induced by diabetes. The marked inhibition of TG output by livers from alloxan-diabetic rats may, in part, be responsible for the fatty liver commonly seen in acute insulin deficiency. Clearly, the release of TG is not proportional in any simple fashion to TG content of the liver, since release of TG was inhibited in livers obtained from diabetic rats even though the neutral fat content of such livers is greater than normal.[1] In the intact animal or patient with acute insulin deficiency, the fatty liver one sees is often accompanied by a hyperlipemia (hypertriglyceridemia). At first glance it would appear that the diabetic hypertriglyceridemia is not compatible with a decreased net release of TG by the liver. A reasonable explanation of this apparent dichotomy may be that more NEFA is available to the liver in the intact diabetic than in the normal animal. The release

of TG by the liver appears to be proportional to the availability of NEFA[3, 9] in addition to any effects of hormones,[4] drugs, or toxic agents[3] on the hepatic TG transport mechanisms. The hypertriglyceridemia in the intact animal may be dependent, in part, on the availability of NEFA to the liver for conversion to TG and for subsequent release of the TG into the plasma, and also dependent, in part, on the rate of removal of triglyceride from the blood stream by liver and other tissues (M. Heimberg, unpublished observations).[10]

TABLE 1. EFFECT OF ALLOXAN DIABETES AND INSULIN ON TRIGLYCERIDE RELEASE AND FATTY ACID UPTAKE BY THE LIVER

| Condition | No. of expts. | TG Release[a] ($\mu$moles/g) | NEFA Uptake[b] (% Uptake/g) | Liver wet weight (g) |
|---|---|---|---|---|
| A. Normal | 8 | $+1{\cdot}39 \pm 0{\cdot}56$ | $4{\cdot}77 \pm 1{\cdot}44$ | $10{\cdot}01 \pm 0{\cdot}57$ |
| Normal, force fed | 2 | $+2{\cdot}50$ | $2{\cdot}96$ | |
| B. Diabetic | 11 | $-1{\cdot}61 \pm 1{\cdot}01$ | $8{\cdot}27 \pm 2{\cdot}78$ | $8{\cdot}22 \pm 1{\cdot}26$ |
| Diabetic, force fed | 1 | $-1{\cdot}87$ | $7{\cdot}75$ | |
| C. Diabetic + insulin (PZI, *in vivo*, 4 U/day)[c] | 5 | $+2{\cdot}53 \pm 2{\cdot}03$ | $5{\cdot}32 \pm 0{\cdot}61$ | $11{\cdot}96 \pm 2{\cdot}05$ |
| D. Normal + insulin (PZI, *in vivo*, 4 U/day)[c] | 6 | $+1{\cdot}57 \pm 0{\cdot}72$ | $5{\cdot}70 \pm 1{\cdot}46$ | $10{\cdot}22 \pm 0{\cdot}76$ |
| E. Diabetic + insulin (crystalline, *in vitro*, infused at rate of 1·8–2·0 mU/ml perfusate/min; primer dose = 0·4 U/ml[d]) | 4 | $-0{\cdot}18 \pm 1{\cdot}67$ | $7{\cdot}71 \pm 2{\cdot}76$ | $8{\cdot}25 \pm 1{\cdot}16$ |
| F. Normal (high NEFA)[e] | 4 | $+3{\cdot}45 \pm 0{\cdot}41$ | | |
| G. Diabetic (high NEFA)[e] | 4 | $+0{\cdot}85 \pm 1{\cdot}18$ | | |

[a] Net releases of TG into the perfusate after 4-hr perfusion. Negative numbers imply net uptake of TG. All figures are means $\pm$ S.D.

[b] Uptake 20 min after NEFA addition.

[c] PZI = protamine zinc insulin. Diabetes was induced by administration of alloxan. After verification of the diabetic state was made by determination of blood glucose, PZI was administered daily to the rats 48, 24, and about 2 hr before surgical removal and perfusion of the liver. Blood glucose levels of the treated animals at time of surgery ranged from 100–200 mg/100 ml.

[d] Crystalline insulin was added to the perfusion medium *in vitro*. If no hepatic catabolism of insulin is assumed, the concentration of the hormone at the end of 1 hr would be about $2{\cdot}4 \times 10^{-6}$ M.

[e] In A–E, 20 mg palmitate as the serum complex (78 $\mu$moles, concentration = 0·6 $\mu$mole/ml) was added at the start of the experiment. In F–G, 40 mg palmitate (156 $\mu$moles, concentration = 1·2 $\mu$moles/ml) was added at the start of the experiment; thereafter palmitate was infused at a rate of 0·167 mg/min (0·65 $\mu$mole/min).

Statistical analysis (values for $t$, P)*

| Condition | NEFA | P | TG | P |
|---|---|---|---|---|
| A vs. B | $t_{17} = 3{\cdot}24$; | <0·01 | $t_{17} = 7{\cdot}00$; | <0·0001 |
| A vs. C | $t_{11} = 0{\cdot}80$; | N.S. | $t_{11} = 1{\cdot}53$; | N.S. |
| A vs. D | $t_{12} = 1{\cdot}19$; | N.S. | $t_{12} = 0{\cdot}53$; | N.S. |
| B vs. C | $t_{14} = 2{\cdot}31$; | <0·02 | $t_{14} = 5{\cdot}59$; | <0·0001 |
| B vs. E | $t_{13} = 0{\cdot}35$; | N.S. | $t_{13} = 2{\cdot}04$; | <0·05 |
| B vs. G | | | $t_{13} = 3{\cdot}78$; | <0·01 |

* Based on a one-tailed table; N.S. = not significant.

The inhibition of net release of TG by the liver from alloxan-diabetic animals may result from an increased oxidation of fatty acids, a decreased rate of esterification of fatty acid to triglyceride, an inhibition of outward transport of TG in the very low-density lipoproteins, or any combination of these factors. This decreased release of TG may also be related to a diminished rate of synthesis of lipoprotein protein. It has been well documented that fatty acid oxidation is increased in livers from diabetic animals.[11] In the experiments reported here, the rapid uptake of NEFA and the inhibition of TG output by the livers from diabetic animals was accompanied by an accelerated rate of formation of ketone bodies (M. Heimberg, unpublished observations). Effects of alloxan diabetes and insulin on TG formation, lipoprotein synthesis, and outward hepatic transport of TG are under investigation.

The nonesterified fatty acid levels in the blood of diabetic animals are higher than in normal animals.[11] A considerable proportion of this supply may be converted to triglyceride even though NEFA oxidation is accelerated. Much of this TG may then be retained within the diabetic liver, giving rise to the fatty liver, while a fraction of the hepatic TG may be released into the blood. It is necessary to note, however, that the livers from normal rats released much more TG than did livers from diabetic animals when the livers were exposed to equivalent amounts of NEFA at low or high concentration of NEFA in the medium.

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## Effect of thalidomide on rat liver regeneration and diaphragm carbohydrate metabolism

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THE implication of thalidomide (DL-N-(2,6-dioxo-3-piperidyl)phthalimide) in human fetal growth and developmental abnormalities such as phocomelia, has led to a variety of researches in mammalian and avian species. Teratogenic effects were described by several workers for the rabbit and mouse, among other species; but resorption to the exclusion of any malformations was observed in the rat, as was also the case with the monkey. DiPaolo[1] pointed out that when the drug was administered to the pregnant mouse before limb bud formation, the toxic effects became quite prominent. The metabolism of thalidomide in the rat has been shown to be rapid[2] and, in contrast to earlier reports, abnormalities in rat fetuses were reported by King and Kendrick.[3]

In the light of the damaging action of thalidomide, especially when administered prior the to advent of the limb buds, it was thought that the agent might elicit a definite effect on the highly proliferative