Note

Triacylglycerol Release by the Perfusion in Situ of Fatty Liver Induced by an Amino Acid Imbalance†

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In a series of studies on the mechanism for inducing fatty liver of the rat fed an amino acid-imbalanced diet containing 8% casein supplemented with 0.3% DL-methionine, we have previously investigated the transport of TG from the liver to the plasma, which has been assumed to be one of the causes of fatty liver induction.

In these studies, in vivo experiments using Triton WR-1339 to block the removal of TG from the plasma and determination of the level and composition of circulating serum lipoproteins were carried out. These results suggested that this type of fatty liver was not due to the inhibition of the transport of hepatic TG to the plasma.

In the present study, the secretion of TG (total and VLDL) from the liver was again investigated, using liver perfusion in situ as a different experimental system, we also attempted to observe the secretion of hepatic TG in orotic acid-treated rats for comparison, because orotic acid is known to lead to a fatty liver by blocking the transport of hepatic lipid into the blood.

Male rats of the Wistar strain weighing about 130 g were used. Before being placed on the experimental diets, the rats were fed for 5 days on a commercial stock diet (Type MF, Oriental Yeast Co., Ltd., Tokyo).

Experiment 1: Amino acid imbalance-induced fatty liver. The rats were divided into two groups of 5 animals each. The experimental diets for each group were as follows:

Group I, 8% casein diet (control)
Group II, 8% casein diet + 0.3% DL-methionine (imbalanced)

The diet composition is shown in Table I. The conditions for feeding the rats were similar to those previously used. Experimental diets and water were given ad libitum for 10 days.

Experiment 2: Orotic acid-induced fatty liver. The rats were divided into two groups of 5 animals each. The experimental diets for each group were as follows:

Group I, 20% casein diet (control)
Group II, 20% casein diet + 1% orotic acid (orotic acid)

The diet composition was similar to that used by Pottenger and Getz. Experimental diets and water were given ad libitum for 7 days.

Liver perfusion. The animals used for the perfusion experiment were anesthetized by intraperitoneal injection of sodium pentobarbital (5 mg/100 g body weight). The livers were perfused in situ by the method of Yagasaki and Kametaka, using a modified Mortimore apparatus built by the authors in this laboratory. The perfusion medium consisted of Krebs-Ringer bicarbonate buffer that contained washed bovine blood cell (25%, v/v), bovine serum albumin (2% w/v), glucose (10 mm), a mixture of amino acids (3.6 mm) and oleic acid (1.6 mm). The oleic acid was dissolved beforehand in 2% albumin solution (4.5 mg of oleic acid/ml of 2% albumin solution). The buffer, containing albumin, was passed through a Millipore filter (pore size 0.45 μ) prior to adding the washed blood cells. Fresh bovine blood cells obtained from a slaughterhouse in Utsunomiya were prepared by the method of Exton and Park. The perfusion medium was aerated with 95% O₂-5% CO₂ and the flow was kept constant at 8 ml per min per liver. The initial volume of the perfusion medium was

<table>
<thead>
<tr>
<th>Casein (%)</th>
<th>Sucrose (%)</th>
<th>Corn oil (%)</th>
<th>Salts (%)</th>
<th>Vitamins (%)</th>
<th>Choline-Cl (%)</th>
<th>DL-Methionine (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.00</td>
<td>82.40</td>
<td>5.00</td>
<td>4.00</td>
<td>0.45</td>
<td>0.15</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Group I, 8% casein diet.
Group II, 8% casein diet + 0.3% DL-methionine.

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Abbreviations: TG, triacylglycerol; VLDL, very low density lipoprotein.
Table II. Effect of Feeding an Amino Acid Imbalance on Body Weight, Liver Weight and Liver Lipid

<table>
<thead>
<tr>
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<th>Liver</th>
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<tbody>
<tr>
<td></td>
<td>Final body weight (g)</td>
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<tr>
<td>8% casein</td>
<td>136.2 ± 4.01</td>
</tr>
<tr>
<td>8% casein +0.3% DL-Met</td>
<td>162.9 ± 5.0*2</td>
</tr>
</tbody>
</table>

1 Mean ± SEM (n=5).
2 An asterisk indicates that the difference from control group is significant (p < 0.05).

80 ml, but 15 ml were flushed through the liver and discarded before closed circulation was established (zero time).

Analytical method. VLDL was isolated by flotation in an ultracentrifuge. The determination of TG in the perfusate plasma and VLDL were carried out as described in a previous paper.3) The liver lipid was determined gravimetrically.

Statistical analysis. The statistical significance of differences between values was analyzed by Student's test.

Amino acid imbalance-induced fatty liver (Experiment 1). The effect of feeding the amino acid-imbalanced diet on the body weight, liver weight and total lipid (Table II) was essentially identical with the published data.2) The effect of feeding the amino acid-imbalanced and the orotic acid diets on the release of TG from the liver is illustrated in Fig. 1. As shown in Fig. 1(A), the release of TG in the imbalanced-diet group was significantly higher than that of the control group throughout the perfusion time, indicating that the induction of this type of fatty liver was not due to the inhibition of transport of TG from the liver.

This result is considered to support the previous assumption in which the effect of triton WR-1339 and the analysis of serum lipoprotein were investigated 2,3) It is quite possible that, in this fatty liver, TG was accumulated by an increased hepatic lipogenesis8) and, furthermore, the release of TG into the plasma was accelerated.

Orotic acid-induced fatty liver (Experiment 2). The development of fatty liver was clearly observed in the orotic acid group (control: 13.3 ± 0.7: orotic acid: 28.3 ± 2.1: % dry basis), as reported by an other investigator.9) The release of TG in the orotic acid group was significantly lower than that of the control group (Fig. 1(B)), indicating that the liver lipids did not enter the blood at their normal rate. The conclusion obtained from the present study was similar to that of Windmueller and Spaeth,9) although different methods were used for the respective experiments.

The release of VLDL-TG from the liver, in Experiments 1 and 2, is shown in Table III. The results that the release of VLDL-TG was higher in the imbalanced-diet group and lower in the orotic acid group than each control group were the same as those obtained in the TG of the unfractionated perfusate.

The present results, therefore, suggest that the mechanism for fatty liver induction by amino acid imbalance is clearly different from that by orotic acid.

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REFERENCES