Role of glucagon on fatty liver production in birds

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Infusions were made with a syringe pump (model 600-910, Harvard Apparatus Co., Dover, Mass.). Appropriate dilutions of glucagon (lot 258-234 B-167-1, Eli Lilly Co., Indianapolis, Ind.) in glycine buffer (0.02 M, pH 9.5 in saline) were used. They were housed, fed, and anesthetized as previously described (19).

After anesthesia one or two catheters were inserted in the wing veins for the infusions. Another catheter was inserted in a wing artery for removal of blood samples. The arterial blood was collected in Corex centrifuge tubes containing heparin powder and kept in a bath of ice water. After removing an aliquot for blood sugar determination, the blood was centrifuged in a refrigerated centrifuge (−2.0 °C) and the plasma separated. Plasma FFA were determined by a modification of Dole’s method and plasma TGL were measured as glycerol after separation on a CO₂-100 of Florisil. The procedures are those described in previous publications (18, 19). Blood sugar was measured with o-toluidine as described by Hyvärinen and Nikkilä (23).

Liver samples were taken before and at the end of the infusions. The methods for extractions, separation, and determination of the liver lipid fractions have been described (19).

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were prepared immediately before use. Infusion rates were 3.06 ml/hr for the geese and 1.24 ml/hr for the ducks.

Oleic acid was infused as an emulsion using Na oleate and gelatin as the emulsifier and stabilizer. The emulsion was prepared by dissolving 1.45 g NaCl and 2.0 g of gelatin (K and K Laboratories, Inc.) in 120 ml of distilled water at 75°C. The solution was placed in the microattachment of a Waring Blendor with 52 ml of 0.2 M Na oleate. USP oleic acid (19 g) was then added while mixing at low speed for 3-4 min. Final dispersion was achieved by mixing at high speed for 3-4 more minutes. The emulsion was then transferred to a beaker adding saline to adjust the concentration to that needed, taking into account the weight of the animal and the infusion rate. The volumes infused were 22.9 ml/hr for the geese and 5.82 ml/hr for the ducks. pH of the emulsion was 6.1-6.2. The emulsion has been tested in a number of animals in this laboratory. Birds tolerated infusion rates up to 4.0 mEq/kg per hr of oleic acid for 2 hr with minimal or no hemolysis. Upon mixing with plasma the turbidity of the emulsion disappears, presumably by binding of the fatty acid to albumin. The plasma separated from arterial blood samples taken during the infusion of the emulsion was transparent except toward the end when there was a marked elevation of plasma TGL. A plateau of the fatty acid concentration measured by Dole's method was achieved after the first 15-30 min of infusion, and it was possible to maintain a wide range of plasma fatty acid levels by changing the infusion rate. Plasma fatty acid concentration decreases sharply upon discontinuing the infusion. The constancy of fatty acid concentration throughout the infusion period indicates that the infused fatty acid is removed from the blood at the same rate as infused. Tests of the utilization of the infused oleic acid were made in geese by comparing the rate of disappearance of oleic acid-14C injected as emulsion and as albumin complex. The same geese were used for the two tests. The $t_{1/2}$ was 1.6 min for both forms of administration. The incorporation of the fatty acid into the plasma TGL was tested by determining the radioactivity of the plasma TGL, after separation on a column of Florisil. The peak of radioactivity in the TGL fraction occurred at 20 min after injection, both when the oleic acid-14C was injected as emulsion and as the albumin complex. These observations, and the results reported by Bezman-Tarcher (4) with intravenous infusion of an emulsion of oleic acid, indicate that the infused fatty acids are utilized in substantially the same way as the endogenous FFA.

RESULTS

The effects of infusing glucagon and oleic acid were studied in three groups of geese (five geese per group). The geese of the first group received glucagon (0.22 μg/kg per min) for 2 hr. Those of the second group were infused with oleic acid (2.0 mEq/kg per hr), also for 2 hr. The geese of the third group received oleic acid (2.0 mEq/kg per hr) for 2 hr and glucagon (1.0 μg/kg per min) for 30 min. Glucagon infusion started 1 hr after that of oleic acid and ended 30 min before ending the infusion of oleic acid. Mean values of plasma FFA, TGL, and blood sugar for each of the three groups of geese are given in Fig. 1.

Glucagon and oleic acid maintained comparable elevations of plasma FFA throughout infusion. At 2 hr of infusion FFA levels were 1.39 mEq/liter (SE ± 0.08, $P < 0.01$) and 1.18 mEq/liter (SE ± 0.13, $P < 0.01$) above the corresponding preinfusion levels for the geese receiving glucagon and oleic acid, respectively.

The geese receiving oleic acid followed by glucagon showed at 1 hr of infusing oleic acid alone an elevation of FFA of 1.30 mEq/liter (SE ± 0.21, $P < 0.01$). After 30 min of adding glucagon infusion to that of oleic acid, plasma

![Graph showing FFA, TGL, and Blood Sugar levels over time for different treatments.]
TABLE 1. Liver lipids of geese infused for 2 hr with glucagon, oleic acid, and oleic acid plus glucagon

<table>
<thead>
<tr>
<th>Infusion</th>
<th>Phospholipids</th>
<th>Total cholesterol</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Infusion</td>
<td>After Infusion</td>
<td>△ After—Before</td>
</tr>
<tr>
<td>Glucagon</td>
<td>28.4 ± 2.81</td>
<td>28.6 ± 1.52</td>
<td>0.2 ± 1.73</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>24.0 ± 2.69</td>
<td>23.3 ± 1.19</td>
<td>0.7 ± 0.7</td>
</tr>
<tr>
<td>Oleic acid and glucagon</td>
<td>29.4 ± 4.60</td>
<td>32.6 ± 2.54</td>
<td>3.2 ± 5.8</td>
</tr>
</tbody>
</table>

Values are mean ± SE for five geese in each infusion group. Glucagon, 0.22 μg/kg per min. Oleic acid, 2.0 mEq/kg per hr for 2 hr, plus glucagon, 1.0 g/kg per min for 30 min.

FFA showed a further rise of 0.33 mEq/liter (SE ± 0.05, P < 0.01).

Plasma TGL rose slowly in the geese infused with glucagon alone. The highest level reached (30 min after end of infusion) was 21 mg/100 ml (SE ± 9.1) above preinfusion. This elevation was not significant (P = 0.11). In contrast, the geese receiving oleic acid alone showed at the end of infusion a significant elevation of plasma TGL (89 mg/100 ml, SE ± 19.3, P = 0.01). Upon discontinuing the infusion of oleic acid the plasma TGL decreased rapidly. In the geese receiving oleic acid and glucagon, plasma TGL rose significantly during the 1st hr of infusion with oleic acid alone. The mean elevation above the preinfusion level at 1 hr of infusion was 56 mg/100 ml (SE ± 10.5, P < 0.01). When infusion of glucagon was added to that of oleic acid, a sharp decrease of plasma TGL was observed. The mean decrease at the end of glucagon infusion was 23 mg/100 ml (SE ± 1.9, P < 0.01) below the value at 1 hr.

Blood sugar rose markedly in the geese infused with glucagon. Mean elevation at end of infusion was 187 mg/100 ml (SE ± 18.2, P < 0.01). Infusion of oleic acid, on the other hand, caused a decrease of blood sugar. The lowest level (90 min of infusion of oleic acid) was 20 mg/100 ml (SE ± 9.1, P = 0.09) below the preinfusion value. The geese of the third group showed also a decrease of blood sugar during the 1st hr of infusing oleic acid alone. Mean decrease at 1 hr was 24 mg/100 ml (SE ± 3.0, P < 0.01). Upon infusing glucagon, blood sugar rose to a level 101 mg/100 ml above that before starting oleic acid (SE ± 26.5, P = 0.02).

A positive correlation was found in the geese infused with oleic acid, between the elevations of plasma FFA and TGL above their corresponding preinfusion levels. Using the values for 20 geese at 1 hr of infusion of oleic acid alone, the correlation coefficient was r = +0.695 (P < 0.01). A higher correlation (r = +0.921, P < 0.01) was found for the values of five geese at 2 hr of oleic acid infusion. No significant correlation was found between the elevations of FFA and TGL at 2 hr of glucagon infusion.

No significant changes of liver phospholipids or total cholesterol were caused by any of the infusions (Table 1). Liver TGL, however, showed a marked increase in the geese infused with glucagon for 2 hr. Infusion of oleic acid alone caused no increase of liver TGL, but infusion of oleic acid plus glucagon caused an elevation of liver TGL smaller than that observed in the geese infused with glucagon alone.

The effect of infusing glucose and oleic acid is shown in Fig. 2. Glucose was given intravenously at the dose of 225 mg/kg, followed by the infusion, for 30 min, of 25 mg/kg per min. The FFA levels were comparable to those observed in the geese infused with oleic acid alone. Plasma...
TABLE 3. Effect of oleic acid infusion on the liver lipids of anesthetized male mallard ducks

<table>
<thead>
<tr>
<th>Lipid Lipids</th>
<th>Phospholipids</th>
<th>Total cholesterol</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before infusion</td>
<td>After infusion</td>
<td>∆ After</td>
<td>Before infusion</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>98.0 ± 1.75</td>
<td>96.9 ± 0.96</td>
<td>-1.14</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>14.4 ± 0.86</td>
<td>11.0 ± 0.11</td>
<td>-3.4</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>11.0 ± 0.96</td>
<td>11.0 ± 0.00</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Values are means ± SE for five ducks. Oleic acid, 4.0 mEq/kg per hr for 2 hr.

TGL showed a mean elevation of 184 mg/100 ml (SE ± 32.2, P < 0.01), above the mean preinfusion level at 2 hr of infusion. This elevation is higher by 95 mg/100 ml (P < 0.01) above the mean preinfusion level at 2 hr of infusion. The analysis of the liver lipids is presented in Table 3. The changes of plasma FFA, TGL, and blood sugar are comparable to those reported for the geese.

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DISCUSSION

The experiments in geese show that infusions of glucagon and of oleic acid maintaining similar plasma FFA levels and of equal duration have different effects on plasma and liver TGL. Glucagon caused a moderate, nonsignificant elevation of plasma TGL and marked deposition of TGL in the liver; oleic acid caused a marked elevation of plasma TGL but no deposition of TGL in the liver. Comparable results were obtained with ducks. These birds, as previously reported (19), developed a deposit of TGL in the liver when infused with oleic acid alone (P < 0.01, t test for nonpaired variates).

Infusions of oleic acid were also made in anesthetized male mallard ducks. These birds required the infusion of 4.0 mEq of oleic acid per kilogram per hour in order to maintain an elevation of plasma FFA levels similar to that produced by the infusion of 0.5 μg/kg per min of glucagon. Mean values for five ducks are presented in Table 2.

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for the synthesis of TGL in the liver (2, 33). Our results are compatible with this view because the plasma FFA of our geese were maintained at a high level by the continuous infusion of oleic acid. It appears, therefore, that when FFA are available, acute administration of glucose enhances the production and release of TGL by the liver. The various biochemical mechanisms involved in this effect have been recently reviewed by Nikkilä (33). Eaton and Kipnis (10) have reported that high-carbohydrate diets stimulate both the synthesis of TGL and that of the protein moiety of the low and very low density lipoproteins in the rat’s liver. These authors suggest that insulin and glucagon play an important role in producing the changes in TGL and lipoprotein-protein synthesis associated with high-carbohydrate diets. Insulin stimulates TGL and lipoprotein formation whereas glucagon is considered to have the opposite effect. Recent observations by Basso and Havel (3) indicate that the fraction of the FFA taken up by the liver which is released as TGL is considerably smaller in pancreactomized dogs deprived of insulin for 48 hr than in control dogs or in normal dogs receiving an infusion of norepinephrine.

The synthesis of the protein moiety of the lipoproteins is a rate-limiting step in the secretion of TGL by the liver; accordingly, a decrease in the synthesis of the apoprotein results in the development of fatty liver (10, 13). It is currently believed that certain substances produce fatty liver by interfering with protein synthesis (11, 38) and that the fatty liver and low serum lipid levels observed in protein malnutrition are the consequence of reduced apoprotein synthesis (15, 26, 27, 37, 43). Glucagon enhances protein catabolism in the liver, stimulating urca production and gluconeogenesis and inhibiting the formation of certain proteins (6, 16, 29, 32, 35, 39). It is possible that glucagon, by stimulating amino acid breakdown, interferes with the synthesis of the apoprotein (10).

Our experiments do not provide information as to the mechanism by which glucagon inhibits the release of TGL by the liver. However, in view of the evidence reported in the preceding references, we suggest that the decreased release of TGL by the liver, produced by glucagon in our experiments, may be explained by the effect of this hormone on hepatic protein catabolism.

The decrease of blood sugar concentration observed in our geese and ducks, during the infusion of oleic acid, compares with that reported by others in nonanesthetized dogs receiving continuous infusions of sodium oleate (8, 20), and with unpublished results in this laboratory using anesthetized dogs.

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