Mannoheptulose and Fatty Acid Synthesis in the Rat1, 2

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ABSTRACT Since metabolic alterations induced by mannoheptulose appear to affect fatty acid synthesis, the present study was conducted to elucidate this relationship. The results indicate that mannoheptulose suppresses hepatic fatty acid synthesis as indicated by a reduced incorporation of glucose-1C into fatty acids and by a decreased activity of acetyl CoA carboxylase. Fatty acid synthesis was restored by exogenous insulin. Mannoheptulose had no effect on the activity of several other lipogenic enzymes and did not affect adipose tissue fatty acid synthesis. The effect of mannoheptulose in the liver appears to be mediated by glucagon. J. Nutr. 104: 473-477, 1974.

INDEXING KEY WORDS mannoheptulose · fatty acid synthesis · acetyl CoA carboxylase · liver

Ingestion or subcutaneous administration of mannoheptulose (MH), a seven-carbon sugar naturally occurring in avocado fruit (1), induces temporary hyperglycemia in man and in several animal species (2-4). In addition, plasma levels of free fatty acids and ketone bodies rise shortly after MH administration (5). The mechanism by which MH induces these metabolic changes is believed to be partially an inhibition of insulin secretion from the pancreas (6), and a direct stimulation of hepatic gluconeogenesis as indicated by nitrogen balance studies and incorporation of 14C from serine into blood glucose (7). MH also increases steady state concentrations of hepatic citrate, phosphoenolpyruvate, ketone bodies, adenosine mono- and diphosphate, and reduces the levels of adenosine triphosphate and fructose diphosphate (5).

These metabolic alterations would indicate an inhibition of hepatic glycolysis and a stimulation of gluconeogenic processes in MH-treated animals. There is ample evidence demonstrating that fatty acid synthesis is directly related to glycolytic rate and substrate availability (8). Consequently, it would appear likely that metabolic alterations induced by MH would suppress the synthesis of fatty acids. The present study was directed at this problem.

Received for publication October 1, 1973.

1 The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.
2 In conducting the research described in this report, the investigators adhered to the “Guide for Laboratory Animal Facilities and Care,” as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institutes of Laboratory Animal Resources, National Academy of Sciences-National Research Council.
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4 Salt Mixture U.S.P. No. XIV, Nutritional Biochemicals Corp., Cleveland, Ohio. When fed at a level of 2.2 g/100 g diet, it supplied the following per 100 g of diet: retinyl palmitate, 1800 IU; cholecalciferol, 220 IU; and: (in mg) alpha- tocopherol, 11; ascorbic acid, 99; inositol, 11; niacin, 10; choline-Cl, 166; menadione, 5; p-amino benzoic acid, 11; riboflavin, 2.2; pyridoxine HCl, 2.2; thiamine-HCl, 2.2; Ca pantothenate, 6.6; and (in ug) biotin, 44; folic acid, 198; and vitamin B-12, 8.
justment period, a 20% solution of p-mannoheptulose in saline (200 mg/100 g body weight) was injected intraperitoneally. The control animals were injected with a corresponding volume of saline. Where applicable, a solution of zinc-insulin, 2 units/100 g body weight, was given intraperitoneally immediately after the MH injection.

Three hours thereafter the animals were decapitated, blood was collected in heparinized beakers, and livers and epididymal fat pads were quickly excised and collected in ice-cold saline. Liver slices, prepared with a Stadie-Riggs microtone, and fat pads were incubated in 25-ml Erlenmeyer flasks containing 3.0 ml of Krebs-Ringer bicarbonate buffer (pH 7.4), and 30 μmoles of glucose with 0.25 μCi glucose-U-14C. The flasks were gassed for 1 minute with 95% O2-5% CO2 and stoppered with a rubber stopper from which a plastic well was suspended. All flasks were incubated in a shaker apparatus for 2 hours at 37°C at 100 strokes/minute. At the end of the incubation period, substrate incorporation into fatty acids and 14CO2 was measured according to the methods described previously (9, 10).

In vitro effects of MH on glucose oxidation and incorporation into fatty acids were studied by increasing the MH concentration in incubation media. Where applicable, insulin was added at 0.1 unit/ml of medium.

Another portion of liver was homogenized according to the method of Muto and Gibson (11) and the soluble liver supernatant obtained by centrifugation at 105,000 × g for 30 minutes was used to assay for the following enzymes by previously described methods: glucose-6-phosphate dehydrogenase, EC 1.1.1.49 (12), NADP-malic dehydrogenase, EC 1.1.1.40 and isocitric dehydrogenase, EC 1.1.1.42 (13); citrate cleavage enzyme, EC 4.1.3.6 (14); fatty acid synthetase, EC 6.2.1.3 (15) and acetyl CoA carboxylase, EC 6.4.1.2 (16). Protein was determined by the method of Lowry et al. (17) and plasma glucose by the glucose oxidase method (18).

Glucose incorporation into tissue metabolites was expressed as micromoles per gram of tissue in 2 hours, and enzyme activity as nanomoles of substrate utilized per minute per milligram of protein. The significance of differences between means, in tables 1, 2 and 3, was evaluated by the Student’s t test. The data in table 4 were evaluated by the analysis of variance. Differences at \( P < 0.05 \) (df 5/30; \( F = 2.53 \)) were considered significant. Differences between means were further evaluated by the Newman-Keuls test.

### TABLE 1
Effect of mannoheptulose on glucose-U-14C oxidation and incorporation into fatty acids by hepatic and adipose tissue preparations

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Liver</th>
<th>Adipose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CO₂</td>
<td>Fatty acids</td>
</tr>
<tr>
<td>Control</td>
<td>4,517±210a</td>
<td>324±56</td>
</tr>
<tr>
<td>Mannoheptulose</td>
<td>4,150±180</td>
<td>94±18a</td>
</tr>
</tbody>
</table>

1 Mean ± SE from six to eight rats. 1 Indicates significant difference from control values, same metabolite, \( P < 0.05 \).

### TABLE 2
Effect of mannoheptulose and insulin on hepatic fatty acid synthesis from glucose-U-14C, glucose-1-14C or glucose-6-14C

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Glucose-U-14C</th>
<th>Glucose-1-14C</th>
<th>Glucose-6-14C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>361±46</td>
<td>273±37</td>
<td>330±48</td>
</tr>
<tr>
<td>Mannoheptulose</td>
<td>87±24</td>
<td>32±14</td>
<td>45±19</td>
</tr>
<tr>
<td>Insulin</td>
<td>375±59</td>
<td>362±42</td>
<td>335±29</td>
</tr>
<tr>
<td>Mannoheptulose +</td>
<td>320±37</td>
<td>280±30</td>
<td>350±35</td>
</tr>
</tbody>
</table>

1 Mean ± SE from five to seven rats. 1 Indicates significant difference from control values, \( P < 0.05 \).
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TABLE 3

Effect of mannoheptulose and insulin on the activity of several enzymes associated with hepatic fatty acid synthesis

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Control</th>
<th>Mannoheptulose</th>
<th>Insulin</th>
<th>Mannoheptulose + Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose-6-phosphate dehydrogenase</td>
<td>181±20</td>
<td>195±17</td>
<td>178±21</td>
<td>189±17</td>
</tr>
<tr>
<td>NADP-Malic dehydrogenase</td>
<td>115±12</td>
<td>128±10</td>
<td>105±9</td>
<td>119±14</td>
</tr>
<tr>
<td>Isocitric dehydrogenase</td>
<td>948±71</td>
<td>987±60</td>
<td>956±51</td>
<td>914±53</td>
</tr>
<tr>
<td>Citrate cleavage enzyme</td>
<td>68±5</td>
<td>60±12</td>
<td>76±10</td>
<td>63±8</td>
</tr>
<tr>
<td>Fatty acid synthetase</td>
<td>27±8</td>
<td>36±30</td>
<td>29±6</td>
<td>30±7</td>
</tr>
<tr>
<td>Acetyl CoA carboxylase</td>
<td>11±0.9</td>
<td>4±0.5†</td>
<td>13±1.2</td>
<td>12±0.7</td>
</tr>
</tbody>
</table>

† Mean ±SE from five rats. † Indicates significant difference from control values, P < 0.05.

RESULTS

As indicated in table 1, MH reduced fatty acid synthesis in liver by approximately 71%, but had no effect on glucose oxidation. MH did not affect fatty acid synthesis or glucose oxidation in adipose tissue.

The data in table 2 show that MH inhibited hepatic fatty acid synthesis from glucose-U-14C by 76%, from glucose-1-14C and -6-14C by about 88 and 86%, respectively. In every case, the synthesis was restored by exogenous insulin.

The results of the enzyme assays are summarized in table 3. Compared to the control value, MH reduced the activity of acetyl CoA carboxylase by 64%. Again, the activity was fully restored by insulin administration. MH had no effect on the activities of glucose-6-phosphate-, NADP malic-, and isocitrate dehydrogenase, citrate cleavage enzyme or fatty acid synthetase.

The in vitro effects of graded levels of MH on glucose oxidation and fatty acid synthesis are given in table 4. At the level of 1 mg/ml without insulin in the medium, MH reduced glucose oxidation and fatty acid synthesis by 55 and 64%, respectively. The inhibition was even greater when the concentration of MH in the medium was increased to 10 mg/ml. The presence of insulin in the medium failed to restore glucose oxidation or fatty acid synthesis.

DISCUSSION

Administration of MH to the intact rat produces a diabetic-like syndrome characterized by hyperglycemia, ketonemia, elevation of plasma free fatty acids and, as found in this study, an inhibition of hepatic fatty acid synthesis. The decreased incorporation of 14C into fatty acids was accompanied by a parallel reduction in the activity of acetyl CoA carboxylase, the rate-limiting enzyme of fatty acid synthesis in animal tissue (19, 20). In contrast, the other enzymes usually associated with lipogenesis were not affected by MH.

Acetyl-CoA carboxylase, it might be noted, is subject to activation or inhibition by a number of metabolic intermediates. It is activated by citrate (21, 22) and inhibited by palmitoyl-CoA (23) or free fatty

TABLE 4

In vitro effect of mannoheptulose, without or with insulin, on glucose-U-14C oxidation or incorporation into fatty acids by liver slices preparations

<table>
<thead>
<tr>
<th>Mannoheptulose mg/ml medium</th>
<th>No insulin</th>
<th>+Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CO2</td>
<td>Fatty acids</td>
</tr>
<tr>
<td>0</td>
<td>4,745±340†</td>
<td>390±48</td>
</tr>
<tr>
<td>0.01</td>
<td>4,940±287</td>
<td>407±53</td>
</tr>
<tr>
<td>0.1</td>
<td>4,504±312</td>
<td>375±39</td>
</tr>
<tr>
<td>1.0</td>
<td>2,140±240*</td>
<td>141±26*</td>
</tr>
<tr>
<td>10.0</td>
<td>1,076±89*</td>
<td>73±15*</td>
</tr>
</tbody>
</table>

† Mean ±SE from six rats. * Indicates significant difference from control values, same metabolite, P < 0.05.
acids (24). Long-chain acyl-CoA compounds may also decrease the activity of the enzyme indirectly by inhibiting the activity of citrate synthetase, thus depriv- ing the carboxylase of its activator (25, 26). Acetyl-CoA carboxylase is also subject to hormonal control, that is, the activity is inhibited by growth hormone (27), and, as recently reported from this laboratory, by glucagon (28). As a consequence, glu- cagon markedly reduces in vivo and in vitro incorporation of glucose into hepatic fatty acids (28, 29), which, in magnitude, is similar to that reduced by MH. Indeed, administration of MH or glucagon elicits similar metabolic effects. Both compounds stimulate free fatty acid release from adipose tissue, increase ketone production and reduce fatty acid synthesis by reducing acetyl-CoA carboxylase. Thus, the interpretation of metabolic alterations induced by MH observed in this study may be based upon insulin insufficiency or, more specifically, upon hyperglucagonemia. This view is further substantiated by the fact that an infusion of MH into the dog over a period of 1 hour causes a progressive rise in plasma glucose, a fall of plasma insulin to undetectable levels and about a fivefold rise in plasma glucagon (30). It would appear that the regulation of hepatic acetyl-CoA carboxylase by MH or glucagon in- volves some activation–inactivation mechanism analogous to the phosphorylation–dephosphorylation mechanisms known in glycogen metabolism (31–33).

The mechanism by which MH induces high glucagon levels remains obscure. MH apparently inhibits phosphorylation of glu- cose in the pancreatic β-cells, a metabolic event necessary for insulin release (34). The concomitant rise in glucagon can not however, be ascribed to hyperinsulinemia per se, since the infusion of MH plus insulin does not prevent the rise of glucagon (30). Inhibition of glucose phosphorylation by MH in the pancreatic a-cells perhaps is the necessary signal for the glucagon release. The resulting decline in the insulin–glucagon molar ratio thus favors mobilization of nutrient stores. In contrast, admin- istration of insulin, resulting in a rise of the insulin to glucagon ratio, will promote storage of nutrients and will effectively overcome metabolic alterations induced by MH.

The failure of MH to reduce fatty acid synthesis in adipose tissue can not be ade- quately explained. Since it is thought that the inhibition of hepatic fatty acid syn- thesis by MH is mediated by glucagon, and consequently, by cyclic AMP, it ap- pears that such inhibitory mechanisms may be ineffective in adipose tissue. Alterna- tively, fatty acid synthesis in adipose tissue may be controlled by mechanisms different from those observed in hepatic tissue. The in vitro inhibitory effect of MH on fatty acid synthesis (table 4) could be attributed to a severe reduction of glucose phosphorylation in liver slices (34) which, in turn, decreases net glucose uptake. The presence of insulin in the incubation media failed to restore lipogenesis. Since insulin fully restored in vivo fatty acid synthesis, it would appear that this in vitro metabolic effect of MH is of little significance in the intact animal.

LITERATURE CITED

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