STUDIES OF PYRUVATE AND ACETATE METABOLISM IN THE HEREDITARY OBESITY-DIABETES SYNDROME OF MICE*

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The genetic characteristics of a new strain of mice (obob) exhibiting recessive Mendelian obesity have been described (1, 2). Obese members of the strain show adult weights of 40 to 60 gm. (occasionally up to 80 gm.), compared to 20 to 30 gm. for the non-obese animals. It has been shown (2) that on "free choice" experiments obese animals select diets much richer in fat than the non-obese. It was furthermore demonstrated (3) that the mature obese animals exhibit a diabetic syndrome characterized by glucosuria, non-fasting sugar levels of the order of 300 mg. per cent, and insulin resistance. These blood sugar levels are very sensitive to dietary conditions (3, 4), being drastically reduced by fasting and also by high fat and high protein, low carbohydrate diets. Histopathological studies (5) reveal that the obese animals present a centripetal distribution of their depot fat, cutaneous atrophy and ulcers, hypertrophy of the islets of Langerhans, and a tendency toward increased number of basophilic cells in the pituitary. No abnormality was noted as far as the adrenals are concerned. The over-all basal oxygen consumption of the obese members of the strain is smaller than that of the non-obese, in spite of their considerably greater weight (6). Several treatments which did not affect the weight of non-obese animals produced a marked reduction in that of the obese, in particular feeding carbohydrate-free diets, diets containing 50 times the normal amounts of B vitamins, and thyroxine administration (4). While the obese animals are extraordinarily resistant to large doses of insulin, they are sensitive to adrenocorticotrophic hormone, growth hormone, pancreatectomy, and various combinations of endocrine treatments (4).

Because these differences of behavior in a variety of dietary and endo-


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crine conditions must be related to metabolic differences between obese and non-obese mice, it appeared useful to compare the fate of administered acetic and pyruvic acids in obese and non-obese animals. Acetic acid has been demonstrated to be a precursor of lipides (7, 8). Pyruvic acid utilization by the diaphragm (9) and the heart muscle (10) of alloxan-diabetic rats has been found to be impaired. This comparison might therefore be expected to yield evidence of the primary metabolic abnormality leading to obesity and diabetes in these animals.

Methods

A total of 52 non-obese and twenty-four obese animals was used. The age of the animals was in the range of 3 to 6 months. The non-obese animals weighed from 20 to 30 gm., averaging 25 gm. The obese animals weighed between 35 to 75 gm., averaging 55 gm. All animals were kept in individual cages at constant temperature and regular illumination. All animals were normally fed Purina chow. During the acetate feeding experiment, the animals received a "synthetic" diet of the following composition: casein 25 per cent, dextrose 65 per cent, corn oil 2 per cent, salt mixture 4 per cent, cystine 0.2 per cent, and choline chloride 0.1 per cent. The diet was supplemented with the following vitamins per kilo of ration: inositol 1.1 gm., p-aminobenzoic acid 0.2 gm., riboflavin 8 mg., pyridoxine 4 mg., calcium pantothenate 20 mg., niacin 20 mg., and thiamine 4 mg.

Alloxan diabetes was induced in eleven non-obese animals by intravenous injection, in the fasted state, of a dose of 3 mg. per mouse. Permanent glucosuria was taken as the criterion of successful production of diabetes.

Respiration Experiments—The mice were fasted for 14 to 18 hours. They were then injected intraperitoneally with 1.0 mM of C14-carboxyl-labeled sodium acetate per 100 gm. of body weight or 1.0 mM of C14-carboxyl-labeled sodium pyruvate per 100 gm. of body weight. Immediately after the injection they were placed in a metabolism chamber. The expired CO2 was collected and radioactivity determined. During the 1st hour, CO2 was collected every 30 minutes; during the following 2 hours, every hour.

Acetate Feeding Experiment—The mice were given daily, over a period of 3 days, 0.1 mM of C14-carboxyl-labeled acetate per 100 gm. of body weight. The acetate was mixed daily with the synthetic diet consumed by the animals. At the end of the 3 day feeding period, the animals were sacrificed and the liver fatty acids and cholesterol isolated and counted for radioactivity, as described elsewhere (11).

1 The mixture of P. H. Phillips and E. B. Hart (J. Biol. Chem., 109, 657 (1935)), modified by the addition of 0.02 per cent CoCl2, was used.
2 The method will be published in detail elsewhere (K. Guggenheim and R. E. Olson).
**Results**

*Oxidation of Acetate*—Fig. 1 shows the cumulative per cent excretion of C$^{14}$O$_2$ after administration of carboxyl-labeled acetate to non-obese and obese mice. Over the 3 hours, the non-obese mice incorporated significantly more C$^{14}$ into CO$_2$ (82 per cent, standard deviation ±13.8 per cent) than the obese animals (70 ± 9.3 per cent). The probability of significance of the difference $p$ is $<$0.05.

*Lipogenesis from Acetate*—In order to study the fate of unoxidized acetate in the obese mice, both non-obese and obese animals were fed C$^{14}$-carboxyl acetate under the conditions described above and the C$^{14}$ activity of liver fatty acids and cholesterol was examined. The results are given in Table I. It is apparent from these results that obese mice show a much higher content of liver fatty acid than the non-obese; concentrations of liver...
### TABLE I

**Amount and Radioactivity of Liver Fatty Acids and Cholesterol in Non-Obese and Obese Mice**

Means and standard errors.

<table>
<thead>
<tr>
<th>Mice</th>
<th>No. of mice</th>
<th>Daily food intake</th>
<th>Weight of liver</th>
<th>Fatty acids</th>
<th>Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>g.m.</td>
<td>g.m.</td>
<td>C.p.m. per 10^4 c.p.m. given</td>
<td>Mg per 100 gm.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Gm. per 100 gm.</td>
<td>Per mg. Total</td>
</tr>
<tr>
<td>Non-obese</td>
<td>12</td>
<td>27</td>
<td>2.0</td>
<td>1.13</td>
<td>3.25 ± 0.26</td>
</tr>
<tr>
<td>Obese</td>
<td>5</td>
<td>52</td>
<td>3.7</td>
<td>2.29</td>
<td>9.45 ± 1.41</td>
</tr>
</tbody>
</table>

Probability that observed difference is due to chance: 0.01 0.01 0.01 0.4 0.02 0.1

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**CUMULATIVE EXCRETION OF C\(^{14}\)O\(_2\)**

**AFTER ADMINISTRATION OF C\(^{14}\)-PYRUVATE**

Fig. 2. Pyruvate utilization in obese, non-obese, and alloxan-diabetic non-obese mice. The figures beside the curves indicate the number of animals.
cholesterol are similar. The specific activity (counts per minute per mg. of fatty acids and of cholesterol) of these substances is considerably greater in non-obese than in obese animals. This greater specific activity of the lipides in the non-obese animals is more than counterbalanced by the disparity in fat content between obese and non-obese animals, and the over-all incorporation of C\textsuperscript{14} in the total fatty acids is more than twice as great in the obese as in the non-obese animals. By contrast, differences in total activity of liver cholesterol between obese and non-obese animals are not significant (p = 0.4). It can be concluded, therefore, that at least a large fraction of the acetate which the obese mice seem unable to oxidize is converted to fatty acids.

Oxidation of Pyruvate—As is seen from Fig. 2, the oxidation of pyruvate is also decreased in obese mice. After 3 hours, 87 per cent (standard deviation ±12 per cent) of the radioactive carbon of carbonyl-labeled pyruvate injected in non-obese mice had appeared in expired CO\textsubscript{2}, whereas obese mice had oxidized only 79 ± 9.4 per cent. This difference is significant at \( p = 0.05 \). The curve representing the oxidation of pyruvate in alloxan-diabetic non-obese mice is similar to that characteristic of the obese animals, but is further depressed. The alloxan-diabetic mice excreted during the 3 hour interval 73 ± 11.3 per cent of the radioactive carbon, a level highly significantly different from the percentage corresponding to the untreated, non-obese animals (\( p < 0.01 \)).

DISCUSSION

The results presented here show that the obese mice do not catabolize acetate at the same rate as the non-obese animals. The acetate which escapes oxidation finds its way, at least for a large part, into synthesized fatty acids. While the rate of incorporation of radiocarbon per unit weight of liver fatty acids and cholesterol is less in the obese than in the non-obese animals, a finding probably related to the greater fat content and smaller metabolic rate (6) of the former, the much greater rate of lipogenesis from acetate of the obese animals is doubtless of significance in the etiology of this form of obesity. Decreased capacity for lipolysis due to decreased acetate catabolism is a corollary.

Comparison of the pyruvate oxidation curves of untreated and of alloxan-treated non-obese mice furnishes evidence \textit{in vivo} of a block in carbohydrate metabolism in diabetes below the pyruvate stage. Studies carried out on rat and pigeon tissues \textit{in vitro} (9, 10, 12-15) have indicated the existence in diabetic tissues of a metabolic block at or below the 3-carbon stage. This block can be removed by insulin. In the case of the obese mice, there is also some depression in the oxidation of pyruvate (Fig. 2). The statistical significance of this decrease is, however, less
than that observed in alloxan diabetes. The physiological significance may be quite different. Diabetic animals are characterized by an impairment in fat synthesis (16). But this decrease in pyruvate oxidation in obese animals must be viewed in the light of the coexistent decrease in over-all oxygen consumption and increased rate of lipogenesis and thus may well be a consequence of the block of acetate oxidation.

The characteristic biochemical lesion of these obese animals appears to be, therefore, a partial block of oxidation of C₂ fragments, with resulting increased lipogenesis and decreased lipolysis. This partial "genetic block" represents probably the primary hereditary lesion in the syndrome. The mechanism of the etiology of acquired secondary symptoms, diabetic in particular, has been discussed in another publication (4).

The mice in this study were obtained from Miss Margaret M. Dickie of the Jackson Memorial Laboratory, Bar Harbor, Maine, who discovered and developed the strain.

SUMMARY

Studies of pyruvate and acetate metabolism in the hereditary obesity-diabetes syndrome of mice were conducted. It was found that, when fasted obese and non-obese mice were injected with carboxyl-labeled sodium acetate, the non-obese mice excreted significantly (one-third) more labeled CO₂ during the 3 hours following injection than did the obese animals. When obese and non-obese animals were fed labeled acetate for 3 days, over-all incorporation of labeled carbon was more than twice as great in the obese as in the non-obese. By means of carboxyl-labeled pyruvate it was shown that pyruvate oxidation was also depressed in the obese animals, though to a lesser extent than in alloxan-diabetic non-obese mice. The partial block of acetate utilization and resultant increased lipogenesis are considered to represent the primary hereditary biochemical lesion in the syndrome.

BIBLIOGRAPHY