Acetone metabolism in mice: Increased activity in mice heterozygous for obesity genes

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ABSTRACT Mice were found to convert acetone to lactate at appreciable rates. The conversion of acetone to gluconeogenic precursors could provide additional glycolytic intermediates that would allow the more complete utilization of lipid stores and increase survival time during starvation. In mice that were starved for 3 days or were provided with acetone in the drinking water the acetone-metabolizing pathway was induced to levels severalfold normal. Mice heterozygous for obesity-producing mutations, either obese (ob+/+ or diabet es (db+/+) showed induction of the activity of this pathway to a significantly higher degree than did homozygous normal (+/+ mice of the same strain. This more effective conversion of acetone to lactate exhibited by heterozygous mice could account for their prolonged survival on a starvation regimen compared to that of normal homozygous. The rate-limiting step in the pathway appears to be the conversion of acetone to a hydroxylated derivative. The enzyme system effecting this conversion is an NADPH-requiring microsomal oxygenase found in the liver.

Acetone is usually considered to be a nonmetabolizable end product of lipid metabolism that accumulates when there are insufficient glycolytic intermediates to effect the complete oxidation of the acetyl CoA generated in the metabolism of fatty acids. The extent, if any, to which acetone can be further metabolized by mammalian species has remained controversial. In studies with rats injected with 14C-labeled acetone, the labeled carbon was found in cholesterol (1), liver glycogen (2, 3), and various amino acids derived from liver and carcass protein (2, 3). Rudney (4) has suggested that acetone can be metabolized by rats to 1,2-propanediol, which is then converted to formate and an unknown 2-carbon fragment, possibly acetate. Sakami and La Faye (5) have suggested that propanediol phosphate is an intermediate in the metabolism of acetone that can be converted directly to some 3-carbon compound in the glycolytic cycle. Further work on the metabolism of propanediol phosphate established a scheme of metabolic events in which it (and thus acetone) could be converted to pyruvate (6, 7). The putative intermediates include hydroxyacetone, propanediol, hydroxypropionate, lactaldehyde, and lactic acid or possibly their phosphorylated derivatives (7).

The possibility that acetone can be converted to gluconeogenic precursors (pyruvate or lactate) raises the question of how much of a contribution acetone can make during periods of prolonged fasting to provide energy and to prolong survival. Support for a substantial degree of conversion of acetone to gluconeogenic precursors comes from a recent study of acetone production and utilization in normal and obese humans during starvation ketosis (8). These studies revealed that only 2–30% of the acetone produced from the metabolism of fatty acid was excreted unchanged in urine, sweat, and breath, whereas the major portion (25–60%) of the acetone produced was metabolized to other products (CO2, glucose, and amino acids). Assuming that all of the acetone produced during fasting potentially could be converted to glucose, these authors calculated that up to 11% of the total glucose produced during starvation could come from acetone metabolism.

Two mutations in the mouse, ob and db, cause identical obesity/diabetes states when maintained on the same inbred backgrounds (9). Both obesity mutants exhibit typical symptoms of obesity and diabetes (hyperglycemia, hyperinsulinemia, and hyperphagia) and they also have remarkable capacity to utilize their food more efficiently. Under severe caloric restriction, the mutants still gain abnormal amounts of weight and remain obese (9); upon total food deprivation; their large fat stores are utilized more efficiently to prolong survival up to 6 times that of normal mice. In spite of a decreased nonfat body mass (10), upon starvation obese mutants have the unusual ability to utilize their entire lipid reserves totally. Their relative lack of tissue protein seems not to interfere with the supply of gluconeogenic precursors required for the maintenance of glycolysis and total fatty acid oxidation.

This increased ability to withstand fasting extends to phenotypically normal heterozygous mice that have only one dose of either the obese or diabetes genes. Both heterozygotes are able to withstand a fast 2–3 days longer than their normal homozygous littermates (11). An ability to utilize food reserves more completely and efficiently may explain the increased survival time observed in heterozygous and mutant mice upon fasting. Certainly, any capacity to utilize more efficiently the acetone produced by the incomplete metabolism of fatty acids or to convert this acetone into gluconeogenic precursors could provide both the additional glycolytic intermediates required to permit the total utilization of lipid reserves and to prolong survival. This report deals with acetone metabolism in normal and heterozygous mice and establishes some of the variables that regulate acetone metabolism in mice.

MATERIALS AND METHODS
Male retired breeders (+/+) of the C57BL/6J (BL/6) and C57BL/KsJ (BL/Ks) strains were obtained at 7–9 months of age from the Animal Resource Department of The Jackson Laboratory. Known heterozygotes, either diabetic or obese (BL/Ks db+/+ and BL/6 ob+/+), were obtained at the same age from our Mutant Resources Center. All of these mice had been established as heterozygotes by their past breeding records. Mice were housed individually and given commercial mouse chow (Emory Morse Co., Guilford, CT) and water ad lib. Mice to be fasted were placed in clean cages and given only water.

Studies in vivo on the conversion of acetone to other metabolic products (CO2, glucose, and glycogen) entailed injection of [2-14C]acetone (as 55 mM solution; 0.18 mCi/μmol; New England Nuclear; 1 Ci = 3.7 × 1010 becquerels) into mice fasted for 3 days; 1 ml (55 μmol) was given per 100 g of body weight. Expired CO2 was collected in 1 M NaOH (5 ml) at 10-min intervals for periods up to 3 hr. A 1-ml aliquot was taken for assay

Abbreviations: BL/6, C57BL/6J mice; BL/Ks, C57BL/KsJ mice.
of radioactivity with Tritosol liquid scintillation fluid. The extent of incorporation of $^{14}$C into glucose was estimated by converting the blood glucose plus a known amount of carrier glucose to the pentaacetate and recrystallizing this derivative to constant radioactivity. In studies on the incorporation of $^{14}$C into glycogen, mice were fasted for only 24 hr, primed with an injection of unlabeled glucose (2 g/100 ml) to stimulate glycogen synthesis, and then 1 hr later were given [2-$^{14}$C]acetone. Liver glycogen was isolated and purified by standard procedures (12).

Studies in vitro were carried out by using homogenates of livers and kidneys of fed and fasted mice. Tissues were homogenized (10 strokes in a Potter–Elvehjem homogenizer) in 5 vol of buffered isotonic saline (0.01 M phosphate, pH 7.0/0.15 M NaCl) and centrifuged at low speed to remove debris and whole cells. This low-speed supernatant was further fractionated by centrifugation at 7000 × g for 15 min in a Spinco ultracentrifuge. This procedure produced a mitochondrion-free supernatant that retained acetone-metabolizing activity. The high-speed supernatant and microsomal pellet were obtained by further centrifugation (100,000 × g) of the mitochondrion-free supernatant. All cofactors and intermediates were obtained from Sigma. The glucose-6-phosphate dehydrogenase was Sigma type XV from bakers' yeast.

The complete reaction mixture, found optimal for acetone metabolism, consisted of: phosphate buffer, 100 μmol, pH 7.3; NADP, 3 μmol; glucose-6-phosphate, 10 μmol; [2-$^{14}$C]acetone, 5.5 μmol (1 μCi); and glucose-6-phosphate dehydrogenase, 0.5 units, in a total volume of 1.7 ml. The reaction was initiated by the addition of 0.5 ml of the mitochondrion-free supernatant. Incubation, usually for 1 hr, was in a Dubnoff shaking incubator at 35°C. The reaction was stopped by the addition of 2 ml of 10% perchloric acid. After centrifugation, the supernatant was neutralized with 30% KOH. The potassium perchlorate was removed by centrifugation and the entire supernatant was placed on a Bio-Rad AG 1-X8 ion exchange column (0.8 × 20 cm; formate form) and washed with 30 ml of water. Neutral compounds, primarily acetone, came off in this water wash. Conversion products, primarily lactic acid, were eluted from the column in about 6 ml of eluate after 20 ml of 0.5 M formic acid had been passed through the column. Aliquots of each fraction were assayed for radioactivity, and the total conversion was calculated. The reaction rate was linear for 2 hr. After this, the rate fell, possibly because of evaporation of substrate.

### RESULTS

**Studies In Vivo.** Significant amounts of $^{14}$C from [2-$^{14}$C]acetone were recovered as $^{14}$CO$_{2}$ in the 3-hr period after injection into fasted mice of all genotypes. The results were variable among individual experiments, and the differences among genotypes were insignificant. On the average, the heterozygotes, either ob/+/ or db/+ , converted 20–40% of the injected acetone to $^{14}$CO$_{2}$ whereas normal mice only converted 15–25%. Significant radioactivity was present in the purified pentaacetate derivative or glucose isolated from plasma. No significant differences in $^{14}$C incorporation into glucose were observed between genotypes in these experiments; however, it was established that carbon from acetone could be converted into some of the carbons of glucose. Based on a mouse blood volume of 2.0 ml, the carbon incorporated into glucose represented about 1% of the injected dose. These same experiments revealed considerable radioactivity in liver glycogen. Under conditions closer to optimal (a 24-hr fast followed by injection of glucose to stimulate glycogen synthesis and then of the labeled acetone), a much larger amount of radioactivity was incorporated into liver glycogen of mice of all genotypes. Mice of the BL/6 strain incorporated somewhat more radioactivity into glycogen than did mice of the BL/Ks strain (Table 1). Regardless of the strain, heterozygous mice, either db/+ or ob/+, incorporated about 50% more radioactivity into glycogen than did normal (+/+) homozygotes.

**Identification of Acetone Metabolites.** Whole homogenates of liver, but not kidney, converted significant amounts of [2-$^{14}$C]acetone to nonvolatile radioactive compounds when fortified with a source of reduced NADP and incubated in the presence of oxygen. These compounds were initially separated from unreacted acetone by evaporation. Subsequently it was found that the major product was acidic and could be retained on an ion exchange column (AG 1) while neutral substances, primarily the unreacted acetone, passed through with the water wash. The acidic component could then be eluted as a sharp single peak with 0.5 M formic acid. Tests of the eluate for glycals (ceric nitrate) and ketones (2,4-dinitrophenylhydrazine) were negative, thus ruling out propanedol, pyruvate, and hydroxypyruvate as potential intermediates. A test of the acidic fraction for bound phosphor was positive. Treatment of the

**Table 1. Conversion of [2-$^{14}$C]acetone to glycogens**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Conversion*</th>
</tr>
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<tbody>
<tr>
<td>BL/6:</td>
<td></td>
</tr>
<tr>
<td>+/+</td>
<td>1.20 ± 0.14</td>
</tr>
<tr>
<td>ob/+</td>
<td>1.80 ± 0.13*</td>
</tr>
<tr>
<td>BL/Ks:</td>
<td></td>
</tr>
<tr>
<td>+/+</td>
<td>0.75 ± 0.16</td>
</tr>
<tr>
<td>db/+</td>
<td>1.13 ± 0.21*</td>
</tr>
</tbody>
</table>

* Shown as μmol of [14C]glycogen synthesized per g of liver per hr, as mean ± SEM. The number of animals is shown in parentheses.

* For difference from normal zygote, $P < 0.01$ (Student’s t test).

**Table 2. Conversion in vitro of acetone to lactate: Activity of various subcellular fractions**

<table>
<thead>
<tr>
<th>Tissue fraction</th>
<th>Activity</th>
<th>μmol/g liver</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Whole homogenate</td>
<td>3.08</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>2. Mitochondrion-free supernatant</td>
<td>2.74</td>
<td>89.0</td>
<td></td>
</tr>
<tr>
<td>3. 100,000 × g pellet</td>
<td>0.86</td>
<td>27.9</td>
<td></td>
</tr>
<tr>
<td>4. 100,000 × g supernatant</td>
<td>0.12</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>5. Reconstituted 3 + 4</td>
<td>1.90</td>
<td>61.6</td>
<td></td>
</tr>
</tbody>
</table>

Tissue fractionation and assays were as described in text, using the subcellular fractions indicated and 1-hr incubation period.

**FIG. 1.** Formic acid elution profile of nonvolatile radioactive material from AG 1-X8 ion exchange columns. Lactate was determined by quantitative conversion to acetaldehyde which, on reaction with p-hydroxyphenyl, produced a purple color that was assayed at 560 nm (15). O, cpm/ml in elute; *, absorbance of eluate at 560 nm; x, cpm/A unit.
The pernatant as source by the column and recovered colorimetrically tabolite. Tests acid cochromatographed indicated. * and evidence of with In homogenates (1,2-propanediol, lactaldehyde, mediates required to localized 2). The buffer-washed as the the mitochondrion-free supernatant fortified with the standard reaction mixture was inactive, suggesting that the homogenate did have some dialyzable cofactors required for some of the steps in the total conversion of aceto to lactic.

All incubations were carried out with the mitochondrion-free supernatant as source of enzyme. Reaction mixture were modified as indicated. Items 1–7 and items 8–10 represent separate experiments using different supernatants.

* See text for complete reaction mixture.

† Coagulum-free supernatant obtained from a 1:1 whole liver homogenate that had been boiled for 5 min; 0.2 ml was used.

acid eluate with alcoholic barium hydroxide to precipitate the organic phosphates precipitated less than 2% of the total radioactivity. The amount of this phosphorylated compound was small and no further effort was made to identify it.

After evaporation to dryness, the formic acid eluate could be rechromatographed through another AgI ion exchange column and recovered again as a single peak (Fig. 1). L-Lactic acid cochromatographed with the unknown radioactive metabolite. Tests of the acid eluate fractions for lactic acid both colorimetrically (13) and enzymatically (with lactic dehydrogenase) were positive. The concentration of lactic acid measured by the p-hydroxydiphenyl assay (A at 560 nm) was proportional to the radioactivity in every case. When mixed with pure lactic acid and neutralized with lithium hydroxide, the eluate formed a lactic acid salt that was readily crystallized to constant radioactivity. In all cases, >90% of the nonvolatile radioactivity was associated with this peak identified as lactic acid. There was no evidence of the accumulation of any other nonvolatile intermediates (1,2-propanediol, lactaldehyde, or pyruvate).

Characteristics of the System In Vitro. The enzymes required to convert acrylic to lactic acid were active in whole homogenates of liver when fortified with a source of NADPH and incubated in air. All of the enzymes required were later localized in the mitochondrion-free supernatant fraction (Table 2). The buffer-washed 100,000 x g pellet had some activity but was only 28% as effective as the mitochondrion-free supernatant. Reconstitution of this pellet with high-speed supernatant restored much activity, but these mixtures were not as active as the unfraccionated mitochondrion-free preparation. No activity was found in the high-speed supernatant alone.

The addition of NADPH or, better, of an NADPH-generating system was essential for activity (Table 3). The addition of NAD, NADH, ATP, and Mg** or of boiled supernatant as a source of unidentified cofactors produced no further stimulation over that seen with the complete reaction mixture. No conversion was obtained in the absence of oxygen. The pH optimum was sharp, occurring at 7.3, and only 50% of the activity was seen at pH 7.2 or 7.6. No intermediates in this conversion were found. The addition of NAD or NADH did not increase the rate or extent of lactate formation, suggesting that, if pyruvate were an intermediate, endogenous cofactors contained in the homogenate must have been sufficient to convert any pyruvate to lactate rapidly. Dialyzed mitochondrion-free supernatant fortified with the standard reaction mixture was inactive, suggesting that the homogenate did have some dialyzable cofactors required for some of the steps in the total conversion of aceto to lactic.

Table 4 presents the rates of aceto conversion to lactic in liver homogenates from normal and heterozygous mice, both fed and fasted. Fed mice had appreciable conversion rates. No significant differences were seen between fed mice of either genotype of either strain. After a 3-day fast, the activity of the pathway was induced 2- to 3-fold in all mice of each genotype. The largest induction was seen in db/+ heterozygotes which converted aceto to lactic about 50% more efficiently than did normal (+/+ +) BL/Ks mice. This increase over that seen in normal mice was not seen in BL/6 ob/+ heterozygotes fasted for only 3 days. However, when the period of fast was increased to 6 days, a significant increment in the activity of the ob/+ heterozygotes over that seen in normal BL/6 mice was observed. Most BL/Ks mice of either genotype failed to survive the 6-day fast.

The relatively long periods of fasting required for maximal induction of this system suggest that the mice must be exposed to some agent that builds up on fasting, possibly aceto itself, before any appreciable induction occurs. To test this, aceto was administered in the drinking water for a period of 3 days at concentrations up to 2.5% (administration of aceto in the drinking water at concentrations of up to 5% had no apparent detrimental effects on the mice; because water consumption decreased at a concentration of 5%, a maximal aceto concentration of 2.5% was used in these studies).

Aceto (1%) administered to fed mice caused the activity of the pathway to more than double after 24 hr of treatment in both strains and genotypes of mice (Fig. 2). No further increase was observed in normal mice of each strain when treatment was extended up to 3 days, but both types of heterozygotes increased the activity of the aceto-metabolizing pathway slightly with the additional 2 days of treatment. Increasing the aceto concentration to 2.5% caused a larger initial induction in all mice than that seen with 1% aceto. The extent of induction increased still further in both normal and heterozygous mice on continued treatment up to 3 days. By this time the BL/Ks db/+ heterozygotes had attained aceto-metabolizing activity 5 times that seen in mice not treated with ace-
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FIG. 2. Effect of administering acetone in the drinking water on the acetone-metabolizing pathway in normal and heterozygous BL/Ks (Left) and BL/6 (Right) mice. No change in total liver weight or other characteristics was observed with acetone administration.

tone, whereas the increment in BL/6 ob/+ heterozygotes was only 3 times. When the induced activities of both heterozygotes were compared to those in their normal counterparts treated with 2.5% acetone, BL/Ks heterozygotes induced the system better than BL/6 heterozygotes (40% compared to 20%). The induced activities in heterozygotes after 3 days of treatment with 1 or 2.5% acetone were significantly different from activities in similarly treated normal homozygotes (P > 0.05).

**DISCUSSION**

The data reported here confirm the results of several groups of investigators (2-6) who showed that acetone can be converted to CO₂, glucose, glycogen, and amino acids, possibly via a 3-carbon glycolytic intermediate in the glycolytic pathway. The finding that lactate is a major intermediate in this conversion provides a means whereby the acetone produced in fasting mice could be involved in gluconeogenesis. Studies with obesity mutants (obese and diabetes) have shown that both mutants and heterozygotes have a remarkable capacity to withstand fasting (11). The conversion of the acetone, produced from incomplete fatty acid metabolism, to gluconeogenic precursors will provide a source of additional energy as well as a mechanism whereby fatty acids could be more completely oxidized in the presence of limited amounts of endogenous carbohydrate. These data establishing the conversion of acetone to lactate document the gluconeogenic potential of acetone produced from fatty acid metabolism. On fasting or treatment with acetone, all mice are able to induce the activity of the acetone-metabolizing pathway. However, heterozygotes have the capability of inducing the pathway significantly better than do normal mice. This additional induction in heterozygotes could provide the extra increment of glycolytic metabolites required to prolong their survival an additional 2-3 days over that observed in normal homozygotes.

When one considers the enzyme system that effects this conversion, two distinctive features are obvious. Both oxygen and reduced NADP are essential for activity. No reaction is seen when assay is performed under nitrogen or with various other cofactors replacing NADPH. This suggests that the system involves an oxygenase, probably one involving a cytochrome P-450 system. The first step in the reaction is presumably the conversion of acetone to hydroxyacetone or dihydroxyacetone. Once this step is completed the subsequent conversion to lactate would follow one of the already established pathways (7). Because no intermediates in the pathway other than lactate were found in our reaction mixtures, it would seem that this oxygenase is the rate-limiting step. Once the hydroxylated acetone is formed, it is rapidly converted to lactate, and intermediates do not accumulate. One would expect that any pathway producing lactate might involve pyruvate as an intermediate. However, I could not demonstrate any accumulation of pyruvate in these studies, nor did additions of NAD or NADH stimulate the conversion. If lactate dehydrogenase is involved in this pathway there must be sufficient enzyme and cofactors in the supernatant to effect the rapid conversion of pyruvate to lactate. Dialyzed mitochondrion-free supernatants fortified with only the NADPH-generating system had no activity with regard to lactate formation, suggesting that this loss of activity could represent the removal of cofactors essential to the conversion of acetone to lactate. The 100,000 × g pellet had marginal activity, indicating the need for enzymes contained in the high-speed supernatant to effect the complete conversion.

The rather long and variable period (depending on the inbred strain) of fasting required to induce the acetone-metabolizing system suggests that a sufficient concentration of some inducing substance must accumulate before the system becomes fully activated. The data reported here suggest that acetone could be the inducing agent because it produced rapid increases in the system when administered in the drinking water. Although all mouse genotypes tested induced the acetone-metabolizing pathway with either fasting or acetone treatments, it is of special interest that both types of heterozygotes (db+/+ and ob+/+) induced it to a greater extent with both treatments than did normal (+/+) homozygotes of the same strain. The extra increment in activity was especially evident in the diabetes heterozygote (db+/+) of the BL/Ks strain. It is significant that this heterozygote (BL/Ks db+/+) has the largest increase in fasting survival time (3-4 days) compared to normal BL/Ks +/+ homozygotes, whereas either BL/6 db+/+ or BL/6 ob+/+ heterozygotes exhibit only a 1- to 2-day increment in survival time (11). Both diabetes and obese homozygous mutants on the BL/Ks background are characterized by a severe diabetes that is life-shortening, rather than just the severe obesity and mild diabetes that are typical of these same mutants on the BL/6 background. This suggests that this particular gene-background interaction produces a synergistic effect which magnifies both the beneficial effects produced in the heterozygote by a single dose of the mutant gene as well as the deleterious effects caused by two doses of the same mutation. A knowledge of gene-background interactions and their effects on metabolic pathways in these heterozygotes should greatly enhance our understanding of obesity and diabetes.

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