Urinary Excretion of 2-Oxo Acids Is Greater in Rats with Streptozotocin-Induced Diabetes

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Summary 2-Oxo acids derived from amino acids, glucose, and fatty acids are key intermediates in energy production. During diabetes, energy production is known to be lower than in healthy individuals. However, it was unknown whether the production of 2-oxo acids is impacted by diabetes. In the present study, I compared the quantities of 2-oxo acids (pyruvic acid, oxaloacetic acid, 2-oxoglutaric acid, 2-oxoadipic acid, 2-oxoisovaleric acid, 2-oxo-3-methylvaleric acid, and 2-oxo-4-methylvaleric acid) excreted in the urine of normoglycemic control rats and rats with streptozotocin-induced diabetes, which reflect the quantities of unused 2-oxo acids in the body. Greater urinary excretion of unused 2-oxo acids thus implies an impairment in energy production. The respective quantities of urinary pyruvic acid1 oxaloacetic acid (measured together), 2-oxoglutaric acid, 2-oxoadipic acid, 2-oxoisovaleric acid, 2-oxo-3-methylvaleric acid, and 2-oxo-4-methylvaleric acid in the diabetic rats were 2.0- (p<0.0001), 2.5- (p<0.0001), 1.5- (p=0.008), 7.6- (p<0.0001), 6.1- (p<0.0001), and 2.1-fold (p<0.0001) greater than in the control rats per 1 g food intake. Thus, the biggest differences were observed in 2-oxoisovaleric acid (a catabolite of valine) and 2-oxo-3-methylvaleric acid (a catabolite of isoleucine). These findings indicate that energy production in the body is suppressed under diabetic conditions.

Key Words 2-oxo acid, streptozotocin diabetes, rat, urine, vitamin

Materials and Methods

Chemicals. 2-Oxoglutaric acid (2-OGA, molecular weight [MW]=146.1), oxaloacetic acid (OXAA, MW=132.1), 2-oxoisovaleric acid (2-OIVA, MW=116.1), pyruvic acid (PyA, MW=88.1), 2-mercaptoethanol, and sodium hydrosulfite were purchased from Wako Pure Chemical Industries, Ltd. Corn oil was obtained from Ajinomoto (Tokyo, Japan). A mineral mixture (AIN-93-G-MX) (3) and a vitamin mixture (AIN-93-VX) (3) were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). All other chemicals were of the highest purity available from commercial sources.

Derivatization of 2-oxo acids with DMB. 2-Oxo acids were derivatized using the method of Shibata et al. (1, 4).

Animals and diets. This study was conducted according to the guidelines for the care and use of laboratory animals and approved by the Ethics Committee of the University of Shiga Prefecture (approval number, No. 27-3).

Male Wistar rats (5 wk old) were obtained from CLEA Japan, Inc. (Tokyo, Japan) and housed individually in metabolic cages (CT-10; CLEA Japan, Inc.). The animal room was maintained at ~22 C, with a humidity of ~60% and a 12/12 h light-dark cycle (lights on, 06:00–18:00). Rats initially had free access to a nutritionally balanced diet provided by CLEA Japan, Inc. (Tokyo, Japan) and water ad libitum. After a 7-day adaptation period, rats were randomly assigned to either the normoglycemic control group or the streptozotocin-diabetic group.
complete diet (20% casein diet) (5) for 7 d, while they acclimated to their environment. After acclimation, rats were divided into two groups (control group, \( n = 6 \); diabetic group, \( n = 7 \)). Rats in the diabetic group were intraperitoneally injected with streptozotocin (STZ) (70 mg/kg body mass) in 0.5% NaCl (pH adjusted to 4.4 using 0.1 mol/L citrate) and the control group was administered with vehicle, following which they were housed for 21 d. The blood glucose concentrations of control and STZ-diabetic rats on the last day of the experiment were 1.107±0.002 mg/mL and 4.783±0.013 mg/mL, respectively.

Body mass and food intake were measured daily at ~09:00, and food and water were renewed at that point. Twenty-four-hour urine samples were collected on the last day of the experiment (09:00 on day 20 to 09:00 on day 21) into bottles containing 1 mL 1 mol/L HCl and then stored at -25°C until use.

2-Oxo acid-radar chart. To construct the 2-oxo acid-radar chart, the relative percentages of each 2-oxo acid for the STZ-treated vs. the control rats were calculated.

Statistical analysis. Statistical significance was determined using Student’s t-test. \( p < 0.05 \) was considered to be statistically significant. All statistical analyses were
performed using GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA).

**Results**

*Food intake and body mass*

Figure 1 shows the daily food intake and body mass changes in the control and diabetic rats. The food intakes and body masses were very similar between the two groups until the fifth day of the experiment. The food intake in the diabetic rats became higher than in the control rats from the 8th day of the experiment, but there was no additional gain in body mass. The total food intakes of the control and STZ groups were 373.2 ± 3.6 g/21 d and 547.8 ± 7.7 g/21 d, respectively (p < 0.0001). The food efficiency ratios (body mass gain over 21 d (g)/total food intake/21 d (g)) were 0.427 ± 0.007 and 0.110 ± 0.009, respectively (p < 0.0001).

*Urinary 2-oxo acid content*

As shown in Fig. 2, urinary excretion of oxaloacetic acid + pyruvic acid (2.0-fold), 2-oxoglutaric acid (2.5-fold), 2-oxoadipic acid (1.5-fold), 2-oxoisovaleric acid (7.6-fold), 2-oxo-3-methylvaleric acid (6.1-fold), and 2-oxo-4-methylvaleric acid (2.1-fold) was higher in the STZ group than in the control group. The values were compared per 1 g food intake, because food intake was different between the groups.

**2-Oxo acid-radar chart**

The urinary 2-oxo acid-radar chart for control and diabetic rats is shown in Fig. 3. Diabetes was associated with greater urinary excretion of all the 2-oxo acids.

**Discussion**

2-Oxo acids derived from amino acids, glucose, and fatty acids are key intermediates in energy production and the urinary excretion of 2-oxo acids reflects the quantities of unused 2-oxo acids in the body. Therefore, greater urinary excretion implies the presence of larger quantities of unused 2-oxo acids in the body, and impairment of energy production. Although the reduced activity of branched-chain 2-oxo acid dehydrogenase [EC 1.2.4.4] in alloxan-diabetic rat liver was reported (6, 7), there are no papers on the amounts of 2-oxo acids under diabetic conditions. These reports (6, 7) predict the increased amounts of 2-oxo acids under diabetic conditions. In the present experiment, the urinary excretion amounts of 2-oxo acids were measured to examine the metabolism of 2-oxo acids in whole animal body. As the results, the urinary excretion amounts of all the 2-oxo acids measured were significantly higher in the diabetic rats than in the control rats, with the biggest differences being observed in 2-oxoisovaleric acid (7.6-fold) (a catabolite of valine) and 2-oxo-3-methylvaleric acid (6.1-fold) (a catabolite of isoleucine). The difference in the urinary excretion of 2-oxo-4-methylvaleric acid (a catabolite of leucine) was not as large (2.1-fold greater in diabetic rats). These three branched-chain 2-oxo acids are metabolized to acyl-CoA by the same enzyme, branched-chain 2-oxo acid dehydrogenase [EC 1.2.4.4] (8), and the metabolism of branched-chain amino acids to branched-chain 2-oxo acids is catalyzed by the same enzyme, branched-chain amino acid aminotransferase [EC 2.6.1.42], in the presence of 2-oxoglutaric acid (9). The branched-chain 2-oxo acid dehydrogenase complex
is generally thought to be the rate-limiting enzyme in the catabolism of branched-chain amino acids (10). If this is true throughout the whole animal body, the magnifications of urinary 2-oxo acid excretion in diabetic rats should be almost the same among the three branched-chain 2-oxo acids. However, as shown in Fig. 3, they were not. Therefore, it is probable that under diabetic conditions catabolic regulation mechanism of branched-chain amino acids is somewhat different compared with the situation under normal healthy conditions. The most probable explanation is that the concentration of catabolic-available leucine in diabetic rats might be lower compared with the control rats. Alternatively, the concentration of anabolic-available leucine, protein-bound leucine, might be higher in diabetic rats.

In conclusion, the present findings indicate that energy production in the body is suppressed under diabetic conditions.

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