Acute and Chronic Treatment of L-Isoleucine Ameliorates Glucose Metabolism in Glucose-Intolerant and Diabetic Mice

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The administration of L-isoleucine (isoleucine) has been shown to induce hypoglycemia in normal rats. However, it remains to be elucidated whether isoleucine can improve the blood glucose level in glucose-intolerant or diabetic animals. In the present study, oral isoleucine significantly reduced the blood glucose level after an oral glucose challenge in normal mice, as well as in glucose-intolerant mice fed a high-fat diet (HFD) and db/db mice, a model of severe type 2 diabetes. Isoleucine treatment significantly augmented the blood insulin level after an oral glucose load in HFD mice, but not in normal or db/db mice, suggesting that its hypoglycemic activity was attributable to both insulinotropic and non-insulinotropic mechanisms. Chronic supplementation of isoleucine in mice on a high-fat/high-sucrose diet significantly reduced insulin release after an oral glucose challenge without any change in glucose tolerance curve, suggesting that isoleucine might have an insulin-sensitizing effect along with its acute hypoglycemic effect. These results indicate that both acute and chronic treatment with isoleucine is beneficial for glucose metabolism in glucose-intolerant and diabetic animals.

Key words L-isoleucine; diabetes; glucose; insulin; hypoglycemia

It is well known that amino acids, one of the three major classes of nutrients, are involved in the metabolism of carbohydrates.1,2 Among various amino acids, L-leucine (leucine) and L-isoleucine (isoleucine) show potent activity for controlling blood glucose. In normal or cirrhotic rats, acute administration of leucine or isoleucine improves the glucose tolerance curve after an oral glucose challenge,3,4 while chronic leucine supplementation reduces obesity and improves glucose metabolism in mice on a high-fat diet (HFD).5 The glucose-lowering activity of isoleucine beyond normoglycemia seems to be relatively mild and becomes saturated as its blood level increases.6 If the glucose-lowering activity of isoleucine in diabetic animals was as strong as in normal animals, this amino acid or another agent with the same target molecule could be a promising candidate as an anti-diabetic agent. Accordingly, we determined the acute effects of isoleucine in glucose-intolerant and diabetic mice. We also investigated the chronic effect of isoleucine supplementation on glucose metabolism in mice fed a high-fat/high-sucrose diet.

MATERIALS AND METHODS

Animals The study protocol was designed to comply with the relevant institutional guidelines and was approved by the Animal Care Committee of Ajinomoto Co., Inc. Male or female C57BL/6J mice (10–16 weeks) and male BKS.Cg-m+/+Leprdb/+Leprdb (db/db mice, 7–8 weeks) were purchased from Charles River Japan (Yokohama, Japan). The animals were maintained in an air-conditioned room (24±1°C) with a 12:12-h light–dark cycle and were given free access to regular chow (CRF-1; Oriental Yeast Co., Tokyo, Japan). In the HFD study, female C57BL/6J mice were fed control chow (D12450B; 4% w/w fat, Research Diet, New Brunswick, NJ, U.S.A.) or an HFD (D12492; 35% w/w fat, Research Diet) for 8 weeks before undergoing a glucose tolerance test. In the high-fat/high-sucrose (HFHS) diet study, female C57BL/6J mice also had free access to drinking water containing 20% sucrose without or with 1 or 2% of isoleucine for 6 weeks. Mice were deprived of food and had access to isoleucine-, sucrose-free water 18 h before undergoing a glucose tolerance test. Since it was reported that female C57BL/6J mice on a HFD develop impaired glucose tolerance after a relatively short period,7 we used female mice for our glucose-intolerance model.

Oral Glucose Tolerance Test Mice fasted for 18 h were given isoleucine orally, which was immediately followed by bolus administration of glucose (1 or 2 g/kg as indicated). Distilled water or 0.5% methylcellulose was given to mice in the control group. To determine the blood glucose and plasma insulin levels, approximately 25 μl of blood was taken from the tail vein at each time indicated. Blood glucose levels were measured by the glucose oxidase method using a Fuji Dri-Chem 5500 autoanalyzer (Fuji Medical Systems, Tokyo, Japan). Plasma was separated by centrifugation and the plasma insulin level was measured by an ELISA kit (Morinaga, Tokyo, Japan). The plasma isoleucine levels were determined by an automatic amino acid analyzer (L-8800; Hitachi, Tokyo, Japan) after deproteinization with 10% trichloroacetic acid.

Statistics Results are expressed as the mean±S.E. Differences between mean values were analyzed by Student’s t-test or Dunnett’s test and p<0.05 was considered significant.

RESULTS

It has already been shown that isoleucine reduces the blood glucose level after an oral glucose challenge in normal Sprague-Dawley rats,3 but not in mice. Therefore, we first confirmed the hypoglycemic activity of isoleucine in normal C57BL/6J mice. Male C57BL/6J mice were given isoleucine...
orally at the doses of 30—300 mg/kg, and then were immediately challenged with 2 g/kg of glucose. As shown in Fig. 1, isoleucine significantly and dose-dependently reduced the blood glucose level, with 16.2% and 24.6% reduction of the glucose area under the curve (AUC) in animals given 100 and 300 mg/kg of isoleucine, respectively. Isoleucine at the dose of 300 mg/kg significantly elevated the plasma isoleucine level from 89.3 ± 6.1 μmol/l to 1220.6 ± 39.1 μmol/l at 30 min and 400.1 ± 49.7 μmol/l at 60 min.

We next studied whether isoleucine could decrease the blood glucose level in HFD mice with impaired glucose tolerance. The glucose AUC was significantly increased by 15.7% in HFD mice compared with control mice (p<0.01), confirming the deterioration of glucose tolerance. Oral isoleucine (300 mg/kg) significantly reduced the height of the glucose tolerance curve in both control mice and HFD mice (Fig. 2A). Interestingly, the plasma insulin level after the glucose challenge only showed a significant increase in isoleucine-treated mice from the HFD group (Fig. 2B), suggesting a different mechanism of hypoglycemic activity in

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**Fig. 1. Effect of Isoleucine Treatment on Glucose Tolerance in Normal C57BL/6J Mice**

After fasting for 18 h, the vehicle (◯) or 30 (●), 100 (▲), or 300 (■) mg/kg of isoleucine (Ile) was given to mice (n=6) by oral gavage just before loading with 2 g/kg of glucose. Serial changes of blood glucose levels (A) and the glucose AUC (B) are shown. Blood samples were collected from the tail vein at each time indicated. *p<0.05; **p<0.01; ***p<0.001 vs. vehicle-treated control mice at the same time.

**Fig. 2. Effect of Isoleucine Treatment on Glucose Tolerance in C57BL/6J Mice on a HFD**

Mice (n=7) were fed control chow (A, left) or an HFD (A, right) for 8 weeks. After fasting for 18 h, the vehicle (◯) or isoleucine at 300 mg/kg (●) was given by oral gavage just before loading with 1 g/kg of glucose. Serial changes of blood glucose levels (A) and the glucose AUC (B) are shown. Plasma insulin levels were determined using blood samples collected from the tail vein (C). *p<0.05; **p<0.01; ***p<0.001 vs. vehicle-treated control mice.
HFD mice from that operating in normal animals.

Although HFD mice showed impaired glucose tolerance, they were not diabetic. We next investigated the glucose-lowering effect of isoleucine in db/db mice, which have severe type 2 diabetes associated with obesity. After a glucose challenge, the blood glucose levels of db/db mice treated with isoleucine showed a dose-dependent decrease and the difference was significant at a dose of 500 mg/kg. However, the insulin $AUC$ did not show any significant difference between isoleucine and vehicle treatment at this dose. Nateglinide, an insulin secretagogue,\(^8\) had no significant effect on blood glucose or on plasma insulin levels in the db/db mice, although it significantly reduced blood glucose levels in normal (data not shown) or HFD C57BL/6J mice\(^9\) at the same dose. These results confirmed that isoleucine effectively regulates the blood glucose level in type 2 diabetic animals without enhancing insulin secretion.

Finally, we studied the effect of chronic isoleucine administration in mice fed a high fat/high sucrose (HFHS) diet. Either 1 or 2% isoleucine was added to the drinking water. HFHS mice rapidly gained weight and showed no difference between the groups with or without isoleucine supplementation (Table 1). There were no differences of water intake, fasting plasma insulin levels, or fecal conditions related to isoleucine supplementation. There was a slight difference of fasting blood glucose between the control group and the 2% isoleucine group. After 6 weeks of treatment, the mice were challenged orally with 1 g/kg of glucose to investigate their glucose tolerance. There was no significant effect of isoleucine on the blood glucose profile after the oral glucose challenge (Fig. 4A). However, the insulin $AUC$ showed a dose-dependent and significant decrease in isoleucine treated-animals (Fig. 4C), suggesting improvement of their insulin sensitivity.

Table 1. Laboratory Data for Mice Fed the High-Fat/High-Sucrose (HFHS) Diet

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1% isoleucine</th>
<th>2% isoleucine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>26.1±0.9</td>
<td>26.5±1.3</td>
<td>24.2±0.8</td>
</tr>
<tr>
<td>Water intake (g/d)</td>
<td>8.6±0.5</td>
<td>8.8±0.5</td>
<td>9.1±0.4</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>99.7±2.6</td>
<td>99.6±1.4</td>
<td>92.3±2.2*</td>
</tr>
<tr>
<td>Fasting insulin (ng/ml)</td>
<td>0.38±0.13</td>
<td>0.30±0.10</td>
<td>0.17±0.04</td>
</tr>
</tbody>
</table>

Values are the mean±S.E. *$p<0.05$ vs. Control.

Fig. 3. Effect of Isoleucine Treatment on Glucose Tolerance in db/db Mice

After fasting for 18 h, the vehicle (○), 50 mg/kg of nateglinide (●), or 100 (▲), 300 (▲), or 500 (■) mg/kg of isoleucine (Ile) was given to mice ($n=9$) by oral gavage just before loading with 1 g/kg of glucose. Serial changes of blood glucose levels (A) and the glucose $AUC$ (B) are shown. The plasma insulin levels of the vehicle, nateglinide, and 500 mg/kg isoleucine groups were determined using blood samples collected from the tail vein, and are shown as $AUC$ (C). $*p<0.05$; $**p<0.01$ vs. vehicle-treated control mice. N.S.; not significant.

Fig. 4. Effect of Chronic Isoleucine Supplementation on Glucose Tolerance in Mice Fed a High-Fat/High-Sucrose (HFHS) Diet

C57BL/6J mice ($n=7$) were fed an HFD and given drinking water containing 20% sucrose without (○) or with 1 (▲) or 2% (■) isoleucine for 6 weeks. After fasting for 18 h with free access to plain water, mice were challenged with 1 g/kg of glucose. Serial changes of blood glucose (A), plasma insulin (B), and the insulin $AUC$ (C) are shown. $*p<0.05$; $**p<0.01$ vs. vehicle-treated control mice.
DISCUSSION

The present study demonstrated that oral isoleucine reduces the blood glucose level in normal animals, in glucose-intolerant HFD mice, and in type 2 diabetic mice. Our findings also suggested that insulin sensitivity was improved in HFHS mice after 6 weeks of isoleucine treatment.

Isoleucine at the dose of 300 mg/kg that had an acute hypoglycemic effect (Figs. 1, 2) increased the plasma isoleucine level from 89.3 μmol/l up to 1220.6 μmol/l at 30 min after administration. In a clinical study, it has been reported that the intake of amino acid drink able to achieve the plasma isoleucine level over 1300 μmol/l is well tolerated without adverse effects. Accordingly, the hypoglycemic effect of isoleucine observed in the animals can be applicable to human without significant side effects.

Since our observations revealed a constant decrease of blood glucose without a consistent increment of plasma insulin in several animal models after acute treatment with isoleucine (Figs. 1—3), we suspected that there were both insulin-secretion dependent and -independent mechanisms involved in the hypoglycemic effect of isoleucine. The insulinotropic effect of isoleucine has already been documented by a study of isolated pancreatic β-cells, in which isoleucine efficiently stimulated insulin release when added together with glutamine. It is possible that glutamate dehydrogenase was allosterically activated by isoleucine and then cause augmented insulin release. On the other hand, the insulin-independent mechanism remains unclear. Nishitani et al. have shown that isoleucine induces rapid translocation of glucose transporters (Glut1 and Glut4) from the cytosol to the plasma membrane in the skeletal muscle of cirrhotic rats. They found no change of the plasma insulin level, although isoleucine treatment significantly decreased the blood glucose level. It has also been demonstrated that isoleucine augments glucose uptake by C2C12 myotubes in vitro as well as by skeletal muscle of normal rats in vivo. These studies suggested the involvement of protein kinase C activation in isoleucine-induced glucose uptake, but the direct target of isoleucine remains to be elucidated.

We considered that acute isoleucine treatment might enhance both glucose uptake by peripheral tissues and insulin release from pancreatic β-cells, with either effect predating in different animal models. It is likely that insulin release would be markedly enhanced in HFD mice treated with isoleucine to compensate for their mildly diminished insulin sensitivity, but not in normal mice because their normal insulin response and isoleucine-induced glucose uptake would be sufficient to prevent hyperglycemia. In diabetic db/db mice that have marked insulin resistance combined with hyperinsulinemia, isoleucine would probably reduce blood glucose levels through improved uptake by peripheral tissues, but not via enhanced insulin secretion from the pancreatic β-cells. It is thought that the insulin-producing ability of pancreatic β-cells would already be near its ceiling in these animals after glucose stimulation alone, since nateglinide (an insulin secretagogue) failed to induce enhanced insulin release or to have a hypoglycemic effect in db/db mice (Fig. 3).

In the chronic study, there was a significant reduction of the insulin AUC, but not the glucose AUC; after an oral glucose challenge in animals supplemented with isoleucine (Fig. 4). Since the animals had access to isoleucine-free water for 18 h before undergoing glucose tolerance test, their blood isoleucine level should have no difference between the groups treated with or without isoleucine. Therefore, we suppose isoleucine supplementation had no direct hypoglycemic effect here unlike the results of the former experiments shown in Figs. 1 and 2. On the other hand, the insulin-saving effect may have been attributable to improvement of insulin sensitivity resulted from long-term supplementation of isoleucine. In fact, chronic supplementation with 1-leucine (an isomer of isoleucine) has also been reported to improve insulin sensitivity in HFD mice. Accordingly, there might be a common target of leucine and isoleucine that influences long-term insulin sensitivity. Obviously, such a target could be very important for the development of pharmacological therapy to treat diabetes and insulin-resistance syndrome. However, additional studies using the insulinclamp technique will need to be performed to confirm the improvement of insulin sensitivity by chronic isoleucine supplementation.

Taken together, our findings that acute and chronic isoleucine supplementation improves glucose metabolism in glucose-intolerant and diabetic mice may provide clues to identify a new drug target. However, further studies are needed to find the molecular target of isoleucine as well as to confirm its hypoglycemic action in humans.

REFERENCES