Trehalose Can Be Used as a Parenteral Saccharide Source in Rabbits

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ABSTRACT Trehalose is a saccharide that possesses no reducing group and so has possible use in parenteral nutrition, especially because it can be stored with amino acids without undergoing the Maillard reaction. To evaluate this possibility, a series of experiments were conducted. The activity of trehalase, an enzyme that metabolizes trehalose to glucose, was measured in rabbit serum and kidney. Conversion of trehalose to glucose and excretion of trehalose in the urine were measured in rabbits administered 10% trehalose intravenously. The effects on nutritional indices as indicators of its use as an energy source were also measured in rabbits infused with 8.23 g · kg⁻¹ · d⁻¹ (4.12 g · kg⁻¹ on d 1) of trehalose for 5 d. Trehalase activity resembled maltase activity, both being high in the renal cortex (2.04 ± 0.71 and 2.93 ± 0.26 μmol · g⁻¹ · min⁻¹, respectively), weak in the medulla, and undetectable in the serum. Serum glucose and insulin concentrations were increased significantly by trehalose infusion. Significant elevations were observed in serum glucose but not insulin levels by maltose infusion. On the other hand, urinary excretion of trehalose (1.1 ± 2.1% of dose) was significantly lower than that of maltose (10.1 ± 4.9% of dose). Similar effects of trehalose and maltose infusions as seen in normal rabbits occurred in rabbits with alloxan diabetes (urinary excretion rate, 3.8 ± 3.0% of the infused trehalose dose and 35.6 ± 9.7% of the infused maltose dose). Nitrogen balance was positive in the trehalose- and glucose-infused normal rabbits with significant difference from the control group infused with saline, suggesting that trehalose was used as an energy source. These results suggest that trehalose has the potential for use as a saccharide source for parenteral nutrition. J. Nutr. 129: 158–164, 1999.

KEY WORDS: • rabbits • trehalase • trehalose • serum glucose • nitrogen-balance

Mono- or disaccharides such as glucose, fructose, xylitol, sorbitol and maltose are used as parenteral carbohydrate sources during periods when oral feeding is not possible due to an operation or for other reasons (Tahara et al. 1990). In parenteral nutritional supplementation, saccharides are usually infused with amino acids, electrolytes and vitamins. Because these saccharides possess reducing groups, they cause a brown discoloration as a result of Maillard reaction when mixed with amino acids for a long time (Fry and Stegink 1982). To prevent this problem, some methods such as storing the saccharides and amino acids in different containers, acetylating the most reactive amino acids (Mukai et al. 1980) and lowering the pH of the solvent (Mori et al. 1993) were developed. However, these procedures create other problems such as adding unnecessary work and inducing angialgia due to the nonphysiological properties of the solutions (Fujikawa et al. 1996, Hasegawa et al. 1995, Ozaki et al. 1995). These problems have been considerably alleviated by methods in which the amino acids and saccharides are mixed together in a dual-chamber bag system (Mori et al. 1991). However, it would be of tremendous value if a new saccharide could be dissolved with amino acids in the same solution without resorting to any special treatment.

Trehalose is a nonreducing disaccharide composed of two d-glucose molecules, which we consume daily, mainly from mushrooms, algae, crustaceans and yeast (Sugimoto 1995). A method of inexpensively producing large amounts of trehalose was recently established (Sugimoto 1995), which is now possible to use on a commercial level. Due to its ability to maintain proteins and nucleic acids stable in cold or dry environments, trehalose recently was used as a stabilizer in foods (Roser 1991), drugs and cosmetics (Kawano 1995). In addition, trehalose can be mixed in a single preparation with other nutrients such as amino acids and electrolytes, and does not participate in Maillard reactions when mixed with amino acids since the molecules do not contain reducing groups. If trehalose were adequately used in the body, it would be of great value as a new saccharide source for parenteral nutrition.

However, little is known about the use of trehalose as a saccharide energy source. Some reports exist concerning the presence of trehalase in organs such as the small intestine and kidney of animals and humans (Dahlqvist 1960, Ruf et al. 1990, Sasai-Takedatsu et al. 1996), and about racial differences in absorption of trehalose caused by deficiency in trehalase when ingested orally (Ushijima et al. 1995a, 1995b). Following intravenous injection of trehalose to rabbits, no trehalose was detected in urine until the concentration of trehalose in blood exceeded 1.59 mmol/L (Riby et al. 1990).
No information is available concerning utilization for energy of intravenously infused trehalose in spite of its properties as a possible element of parenteral solutions. We therefore conducted a study in rabbits to determine whether intravenously infused trehalose is hydrolyzed to glucose and utilized as a saccharide energy source. We first measured the activity of trehalase, the enzyme that catalyzes the hydrolysis of trehalose, in serum and kidney, the latter of which is considered to be the major organ for the metabolism of maltose, another disaccharide (Fuji et al. 1972), to evaluate possible use of trehalose. We then investigated the extent to which trehalose is converted to glucose when intravenously infused and the amount excreted in urine in comparison with maltose. We also investigated trehalose in rabbits with alloxan diabetes in which glucose tolerance is reduced. Having confirmed sufficient hydrolysis of intravenously infused trehalose to glucose, we investigated the effects of trehalose on nutritional indices by continuous infusion with amino acids, electrolytes and vitamins to food-deprived rabbits for 5 d compared with glucose.

### MATERIALS AND METHODS

The protocol complied with the guidelines for the care and use of laboratory animals at Otsuka Pharmaceutical Factory, Inc. (Nanbu, Japan).

**Experiment 1. Measurement of trehalase (EC 3.2.1.28) and maltase (EC 3.2.1.20) activities in the serum and the kidney.** Trehalase and maltase activities were measured by modifying the methods described by Ohneda et al. (1973). Laparotomy was performed on male Japanese White rabbits (Kitayama Labs, Osaka, Japan) under pentobarbital anesthesia (Dainippon Pharmaceutic Co., Ltd., Osaka, Japan). After collecting blood from the abdominal aorta, the kidneys were removed. The blood obtained was centrifuged at 2000 × g for 20 min, and measurements were made in serum. The kidneys were dissected on ice into cortex and medulla after being washed with physiological saline (Otsuka Pharmaceutical Factory, Inc.). A 50-fold homogenate of cortex and a 10-fold homogenate of medulla were prepared with Krebs-Ringer-bicarbonate buffer (the enzyme solutions). Trehalase (α-D-glucopyranosyl α-D-glucopyranoside; Hayashibara Biochemical Laboratories, Inc., Okayama, Japan) and maltose (Maltose Monohydrate; Wako Pure Chemical Industries, Ltd., Osaka, Japan) were dissolved in Krebs-Ringer-bicarbonate buffer to make 0.2 mol/L solutions (the substrate solutions). After preincubating 0.5 mL each of the enzyme solution and the substrate solution at 37°C for 10 min, they were mixed and incubated at 37°C for an additional 60 min. Following incubation, the mixtures were chilled on ice and centrifuged at 2000 × g for 10 min, and the glucose in the supernatant was measured with DRI-CHEM5500 (Fuji Film, Tokyo, Japan). The amounts of glucose produced per liter serum or g tissue were calculated as an index of the enzyme activity.

**Experiment 2. Assessment of utilization of trehalose during 90-min continuous infusion in normal rabbits.** Male Japanese White rabbits (10–13 wk of age; Kitayama Labs, Osaka, Japan) were individually housed in metabolic cages. After overnight food deprivation, the rabbits were continuously infused with either 100 g/L trehalose solution, 100 g/L maltose solution, 50 g/L glucose solution (prepared from 700 g/L glucose solution, Otsuka Pharmaceutical Factory, Inc.), or saline (control) via an auricular vein with an infusion pump for 90 min at a rate of 6.7 mL × kg⁻¹ × h⁻¹ (10 mL × kg⁻¹ × 90 min⁻¹) without other nutrients. Before the infusion, and 30, 60, 90, 120 and 180 min after the start of the infusion, 3 mL of blood samples were collected from the auricular artery on the opposite side, and the serum was isolated and frozen at −80°C until measurement. Urine was collected during the 24 h immediately after start of the infusion, during which the rabbits were food-deprived, and after measuring its volume, a sample was stored at −80°C. The concentrations of glucose (enzyme method: Banauch et al. 1975), trehalose, maltose (high performance liquid chromatography method: modified from Riby et al. 1990), and insulin (RIA2 antibody method: Nomura et al. 1984) were measured. The urine sample was used to measure the concentration of glucose, trehalose, and maltose. Urinary excretion of trehalose and maltose was calculated as percentages of the amounts administered.

**Experiment 3. Assessment of utilization of trehalose during 90-min continuous infusion in rabbits with alloxan diabetes.** Alloxan diabetes was induced by injecting 10- to 13-wk-old male Japanese White rabbits (Kitayama Labs) with 2 mL/kg of 100 g/L alloxan (Sigma Chemical Co., St. Louis, MO) via an auricular vein. From the following evening, the rabbits were individually housed in metabolic cages, and deprived of food until the end of experiment. Two days after alloxan injection, a blood sample was collected from an auricular vein, and serum glucose was determined. The rabbits were then divided into three groups so that the serum glucose concentrations in each group would be the same. The groups were then continuously infused with either 100 g/L trehalose solution, 100 g/L maltose solution or saline (control) for 90 min via an auricular vein at a rate of 6.7 mL × kg⁻¹ × h⁻¹ with an infusion pump. Before the start of the infusion and 90 and 180 min after the start of the infusion, a blood sample was collected and treated as in Experiment 2. Urine was also collected and treated as in Experiment 2.

**Experiment 4. Effect of trehalose on nutritional indices during 5-d continuous infusion in normal rabbits.** Male Japanese White rabbits (10–13 wk of age; Kitayama Labs) were individually housed in metabolic cages. After food was withheld overnight, a silicone catheter (1.0 mm i.d.; Fuji Systems, Tokyo, Japan) was placed in each rabbit for parenteral administration via the right external jugular vein with the tip pointed toward the right atrium. The catheter was then tunneled subcutaneously to exit between the scapulae and connected to a silex-spring apparatus protected by a stainless-steel flexible spring sheath. Oral intake of food and water was not allowed during the study period. The infusion (Table 1) containing either trehalose or glucose, amino acids (Aminiparin; Otsuka Pharmaceutical Factory, Inc.), electrolytes (Otsuka Pharmaceutical Factory, Inc.) and vitamins (Otsuka MV injection; Otsuka Pharmaceutical Factory, Inc.) was continuously administered for 5 d. On d 1, 50% of the infusion was infused while 100% was infused from d 2 onward. The total amount of saccharide administered was 8.23 g × kg⁻¹ × d⁻¹. A group to which all components were administered in the same amounts as

<table>
<thead>
<tr>
<th>Amount infused, unit × kg⁻¹ × d⁻¹</th>
<th>Control</th>
<th>Glucose</th>
<th>Trehalose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trehalose, g</td>
<td>0</td>
<td>0</td>
<td>8.23</td>
</tr>
<tr>
<td>Glucose, g</td>
<td>0</td>
<td>8.23</td>
<td>0</td>
</tr>
<tr>
<td>Amino acids¹, g</td>
<td>3.29</td>
<td>3.29</td>
<td>3.29</td>
</tr>
<tr>
<td>Electrolytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na, mmol</td>
<td>3.84</td>
<td>3.84</td>
<td>3.84</td>
</tr>
<tr>
<td>K, mmol</td>
<td>2.74</td>
<td>2.74</td>
<td>2.74</td>
</tr>
<tr>
<td>Cl, mmol</td>
<td>3.84</td>
<td>3.84</td>
<td>3.84</td>
</tr>
<tr>
<td>Ca, mmol</td>
<td>0.33</td>
<td>0.33</td>
<td>0.33</td>
</tr>
<tr>
<td>Mg, mmol</td>
<td>0.33</td>
<td>0.33</td>
<td>0.33</td>
</tr>
<tr>
<td>P, mmol</td>
<td>0.46</td>
<td>0.46</td>
<td>0.46</td>
</tr>
<tr>
<td>Multivitamins², mL</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Total volume, mL</td>
<td>98.7</td>
<td>98.7</td>
<td>98.7</td>
</tr>
<tr>
<td>Total energy, kJ</td>
<td>55</td>
<td>193</td>
<td>193</td>
</tr>
<tr>
<td>Total nitrogen, g</td>
<td>0.52</td>
<td>0.52</td>
<td>0.52</td>
</tr>
<tr>
<td>NPE/NP, kJ/g</td>
<td>63.3</td>
<td>63.3</td>
<td>63.3</td>
</tr>
</tbody>
</table>

¹ The composition was as follows (g/L): L-leucine, 14; L-isoleucine, 8; L-valine, 8; L-lysine, 10.5; L-threonine, 5.7; L-tryptophan, 2; L-methionine, 3.9; L-phenylalanine, 7; L-cystine, 1; L-tyrosine, 0.5; L-arginine, 10.5; L-histidine, 5; L-alanine, 8; L-proline, 5; L-serine, 3; L-glycine, 5.9; L-aspartic acid, 1; L-glutamic acid, 1.

² The composition was as follows (mg/L): thiamine HCl, 0.975; riboflavin, 1.15; pyridoxine HCl, 1.225; vitamin B12, 0.00125; ascorbic acid, 25; D-biotin, 0.015; folic acid, 0.1; panthenol, 3.5; nicotinic acid amid, 10; vitamin A, 825 (RE); cholecalciferol, 0.00125; tocopheryl acetate, 2.5; vitamin K, 0.5.

³ NPE/N: nonprotein energy/g nitrogen.
TABLE 2
Trehalase and maltase activity in serum and kidney from rabbits (Expt. 1) \(^1\)

<table>
<thead>
<tr>
<th></th>
<th>Trehalase ( \text{mmol} \cdot \text{L}^{-1} \cdot \text{min}^{-1} )</th>
<th>Maltase ( \text{mmol} \cdot \text{L}^{-1} \cdot \text{min}^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney, Medulla</td>
<td>0.15 ± 0.13 (^b)</td>
<td>0.22 ± 0.10 (^b)</td>
</tr>
<tr>
<td>Kidney, Cortex</td>
<td>2.04 ± 0.71 (^a)</td>
<td>2.93 ± 0.26 (^a)</td>
</tr>
</tbody>
</table>

\(^1\) Values are means ± sd, \(n = 3\). Means with no letters in common differ significantly \((P < 0.05, \text{paired } t\text{-test})\).

RESULTS

Experiment 1. Measurement of trehalase and maltase activities in serum and kidney. Neither trehalase nor maltase activity was detected in the serum (Table 2). Trehalase and maltase activities were detected in the kidney, and the activities of both were more than 10-fold greater in the cortex than the medulla.

Experiment 2. Assessment of utilization during 90-min continuous parenteral infusion in normal rabbits. After beginning parenteral infusion of trehalose, the serum glucose concentration rose significantly and peaked at 90 min (Fig. 1A). After completion of the infusion, the serum glucose level decreased, and after 90 min had returned to its preinfusion value. Similar changes were observed in response to maltose infusion, but the proportional increase in serum glucose was less than in rabbits infused with trehalose. The dose of glucose infused was half that of trehalose and maltose. In these rabbits, the serum glucose concentration was significantly greater than baseline only at 60 min after start of the infusion and decreased to the preinfusion value within 30 min after completion of the infusion. Patterns of changes in serum insulin levels due to saccharide infusion were similar to those of serum glucose, but not significant except between the control and

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\(\text{FIGURE 1}\) Serum glucose (A) and insulin (B) concentrations in food-deprived normal rabbits infused with either saline (control), 100 g/L trehalose, 100 g/L maltose or 50 g/L glucose for 90 min (Expt. 2). Values are means ± sd, \(n = 4\). *Significantly different from prevalue \((P < 0.05, \text{Dunnnett’s test})\). Means with no letters in common at the same time points differ significantly \((P < 0.05, \text{Tukey-Kramer test})\).
Means with no letters in common differ significantly (P < 0.05). The serum trehalose concentration was lower than maltose concentration when trehalase-infused groups (Fig. 1B). The serum trehalose concentration was lower than maltose concentration when trehalose or maltose was infused (P = 0.09 at 90 min after the infusion).

Glucose excreted in urine was greater when maltose was infused than when saline or glucose was infused (Fig. 2). There was no difference in glucose excretion between trehalose- and maltose-infused rabbits and among rabbits infused with saline, glucose and trehalose. Urinary excretion of trehalose and maltose differed significantly (P < 0.05). In rabbits infused with maltose, 10.1 ± 4.9% of the dose was excreted in the urine, compared with 1.1 ± 2.1% in those infused with trehalose.

**Experiment 3. Assessment of utilization of trehalose during 90-min continuous infusion in rabbits with alloxan diabetes.** Despite overnight food-deprivation, serum glucose concentrations remained high 2 d after the alloxan injection (Fig. 3A). Serum glucose levels rose further as a result of continuous infusion of trehalose or maltose, but the glucose levels were not significantly different between the trehalose- and maltose-infused groups. The elevated serum glucose concentrations in both groups persisted after completion of administration for at least 90 min. There were no significant differences in serum insulin levels among groups (Fig. 3B).

Urinary excretion of glucose was not significantly different among groups. Urinary excretion of trehalose and maltose was 3.8 ± 3.0% and 35.6 ± 9.7%, respectively, of their respective doses, and the difference was significant (P < 0.05).

**Experiment 4. Effect on Nutritional Indices During 5-d Continuous Infusion in Normal Rabbits.** Since many of the rabbits did not urinate on d 1 after the infusion, measurements were made on d 2. Urinary glucose excretion in the trehalose-infused group did not differ from that in the glucose-infused group, with neither being significantly different from the control group except on d 2 in the glucose group when it was significantly greater (data not shown). Urinary excretion of trehalose was less than 1% of the total dose infused (data not shown).

Body weight loss did not significantly differ among the three groups nor were there any differences in liver or kidney weights (data not shown). The weights of the gastrocnemius muscle were significantly greater in the trehalose- and glucose-infused groups than in the control group (9.62 ± 0.68, 9.70 ± 0.63 and 8.48 ± 0.64 g, respectively). Cumulative nitrogen-balance (N-balance) was positive in the trehalose- and glucose-infused rabbits with significant differences from the control group infused with saline (Figs. 4A and B).

No significant differences were detected in insulin concentrations, total protein, albumin or albumin/globulin ratio (data not shown). Blood urea nitrogen (BUN) was significantly lower in the trehalose group than in the control group (Table 3). Although there were no significant differences among the groups in serum triglycerides and phospholipids (data not shown), nonesterified fatty acid concentration was significantly lower in the trehalose-infused group than the control group (Table 3). The levels of acetocetic acid and β-hydroxybutyrate were lower in both the trehalose- and glucose-infused groups than in the control group (Table 3). Serum Na and Cl concentrations were higher in the trehalose-infused group than in the glucose-infused and control groups (Table 3). No significant differences among the groups in urinary electrolytes and excretion of 3-methylhistidine were observed (data not shown).

**DISCUSSION**

Trehalose, a disaccharide present in a variety of foods, has no reducing group and consequently, does not cause the Maillard reaction, even when mixed and stored with amino acids. Accordingly, it can be formulated with amino acids in a single preparation for intravenous infusion, thus having possible use as a new saccharide source for parenteral solutions. However, for trehalose to be used as a saccharide source for parenteral nutrition, it must be hydrolyzable to glucose after introduction into the body.

To determine whether trehalose is converted to glucose in the body, we measured the activity of the trehalose-degrading enzyme (trehalase) in rabbits, as they are often used in saccharide research. No trehalase activity was detected in the serum of rabbits, and simultaneous attempts to measure maltase activity in the serum were also negative. Maltase activity was detected in the blood of rats (Fujii et al. 1972), but it was not to be found in the blood of rabbits and humans (Ohneda et al. 1973). The results of our study corroborate these findings. In contrast, trehalase activity was found in the kidney, with activity being higher in the cortex than in the medulla, similar to maltase (Ohneda et al. 1973). Based on this enzyme activity in vitro, it was hypothesized that trehalose could be used as a saccharide source for intravenous administration. To determine this, we next assessed its utilization in vivo.

After trehalose was infused into the auricular vein of normal rabbits, the serum glucose levels rose more than when maltose was infused. In contrast, the increase of trehalose concentration in serum after trehalose infusion tended to be less than when maltose was infused. Moreover, the urinary excretion of trehalose was about 1% of the total dose infused (data not shown).

Maltase and trehalase activities are lower than normal in...
kidney brush border membranes from diabetic rabbits (Itoh et al. 1989). We studied utilization of trehalose in diabetic rabbits induced with alloxan. Although changes in serum glucose and insulin levels in diabetic rabbits receiving trehalose or maltose showed different patterns than those observed in normal rabbits, there apparently was greater conversion of trehalose compared with maltose to glucose in the diabetic rabbits. More trehalose was excreted in urine in diabetic rabbits than in normal rabbits, but whereas more than 30% of the dose of maltose was excreted in urine, less than 4% of trehalose was excreted, indicating that trehalose was converted to glucose in sufficient amounts even when glucose tolerance was reduced. However, since urinary excretion of glucose also increased to a considerable degree in diabetic rabbits compared with normal rabbits even in the trehalose group, it is necessary to consider the rate of infusion to determine

![FIGURE 3](image_url)

**FIGURE 3** Serum glucose (A) and insulin (B) levels in food-deprived rabbits with alloxan diabetes infused with either saline (control), 100 g/L trehalose or 100 g/L maltose for 90 min (Expt. 3). Alloxan (2 mL/kg of 100 g/L) was injected to rabbits via an auricular vein to induce diabetes. Values are means ± sd, n = 5. *Significantly different from prevalue (P < 0.05, Dunnett’s test). Means with no letters in common at the same time points differ significantly (P < 0.05, Tukey-Kramer test).

![FIGURE 4](image_url)

**FIGURE 4** Daily (A) and cumulative (B) Nitrogen-balance (N-balance) in rabbits infused with either saline (control), trehalose or glucose for 5 d (Expt. 4). Oral intake of food and water was not allowed during the study period. Rabbits were infused with trehalose or glucose at 8.23 g · kg⁻¹ · d⁻¹ in combination with amino acids, electrolytes and vitamins. Controls were infused with saline instead of saccharide, with all other components being the same. Urine was collected every 24 h except for the first 2 d, where all urine was collected together. Nitrogen-balance was calculated by subtracting total excreted nitrogen from total infused nitrogen. Values are means ± sd, n = 5. Means with no letters in common at the same time points or in the 5-d accumulation differ significantly (P < 0.05, Tukey-Kramer test).
Trehalose as a Parenteral Saccharide Source


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REFERENCES


1 Values are means ± SD, n = 5 for control and trehalose groups and 6 for glucose group. Means in a row with no letters in common differ significantly (P < 0.05).

2 BUN, blood urea nitrogen.

3 NEFA, nonesterified fatty acids.

mine if trehalose can be sufficiently used as a saccharide energy source in the diabetic state.

On the basis of high conversion of trehalose to glucose when intravenously infused, we further examined the effects on nutritional indices by continuous infusion of trehalose vs. glucose for 5 d with other nutrients including amino acids, electrolytes and vitamins to rabbits without other food supply. Trehalose excretion in urine did not increase even when administered for 5 d. No difference existed in glucose excretion between the trehalose- and glucose-infused groups at the infusion rate used in the present experiment. N-balance, which reflects protein catabolism (Wilmore 1983), showed a 46 mmol/kg decrease during the 5-d period in the control group, indicating that protein was catabolized and used as an energy source due to the lack of a saccharide energy source. In contrast, N-balance in the trehalose-infused rabbits did not differ from that in those infused with glucose, confirming that it was adequately used as an energy source. Although no significant differences in protein components in the serum were observed, BUN was significantly lower in the trehalose-infused rabbits than in the control group, indicating that less protein breakdown had occurred. Free fatty acids and ketone bodies mobilized from adipose tissue were significantly lower in the trehalose-infused rabbits than in the control group, indicating that trehalose and glucose were sufficiently used as energy sources and that fat breakdown was prevented. No changes in other components such as creatinine or uric acid that might become a problem were observed.

Infused trehalose was used by rabbits as a saccharide energy source. Species differ in the enzyme metabolizing another disaccharide, maltose (Fuji et al. 1972). It is therefore necessary to further investigate differences in utilization according to species to assess the possibility of using trehalose as a new saccharide energy source for parenteral nutrition in humans.

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