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Alpha-amylase inhibitor increases plasma 3-hydroxybutyric acid in food-restricted rats

T. Doi, H. Yoshimatsu[a], I. Katsuragi[a], M. Kurokawa[a], H. Takahashi[b], A. Motoshio[b] and T. Sakata[a,*]

Department of Internal Medicine I, Faculty of Medicine, Kyushu University, Fukuoka 812; [a]Department of Internal Medicine I, School of Medicine, Oita Medical University, Oita 879-55, Fax +81 975 49 4480, and [b]BIOS Central Research Laboratory of Pharmacology and Pharmacy, Tokyo 180 (Japan)
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Abstract. The effect on energy metabolism of delayed absorption of starch by inhibition of α-amylase was examined by considering levels of plasma glucose and 3-hydroxybutyric acid (3-OHBA) in rats. Addition of α-amylase inhibitor (αAI) to a high starch diet delayed the plasma glucose response after feeding: peak plasma glucose levels in the control group occurred 15 min after feeding, whereas in the αAI group this peak did not occur until 30 min after. The total plasma glucose response was not different between the two groups. Plasma 3-OHBA levels 1 day after food restriction increased approximately five-fold in both groups. After 3 days of food restriction, the αAI group maintained the same level of plasma 3-OHBA as after 1 day of food restriction, while the control group showed significantly decreased levels of 3-OHBA. After 3 days of food restriction, plasma insulin levels were significantly decreased in the αAI group compared with the corresponding levels of the control group and with levels before the restriction. There was no significant difference in body weight between the two groups. These findings suggest that delayed hyperglycemia due to delayed absorption of starch following αAI loading may attenuate insulin secretion, leading to altered metabolism of 3-OHBA during the delayed response to energy deficit.
Key words. α-amylase inhibitor; plasma glucose; 3-hydroxybutyric acid; high starch diet.

Reducing starch absorption by different means such as increased intake of dietary fiber[1] or α-glucosidase inhibitor[2] has been advocated as a possible therapeutic approach to reduce energy intake. Alpha-amylase inhibitor (αAI) is found in common foodstuffs such as wheat and kidney beans[3,4]. It reduces the digestion and absorption of starch, presumably through its inhibitory effect on activity of salivary and pancreatic amylase[5]. Administration of αAI was shown to reduce hyperglycemia and hyperinsulinemia after tube-fed starch loading in rats[6]. These findings suggest that reduced absorption of starch due to αAI may be clinically useful if αAI attenuates hyperglycemia and/or hyperinsulinemia after meals. The commercial availability of αAI opens new possibilities for using inhibition of starch digestion in weight control[7,8]. However, essential data to confirm the efficacy of αAI are scant, because it is not known how αAI affects glucose levels after ad libitum feeding of mixed meals; nor is it clear how the reduced hyperglycemic effect caused by αAI influences overall metabolism after αAI loading.

Plasma levels of 3-hydroxybutyric acid (3-OHBA), a ketone body, are known to increase during fasting[9,10] and to suppress food intake in rats[11]. In humans, elevation of plasma 3-OHBA during food restriction suppresses feelings of hunger[12]. This attenuation of hunger due to increased plasma 3-OHBA has been used to aid food restriction as a dietary therapy[12]. Plasma levels of 3-OHBA are used as an indicator of body insulinogenic activity: there is a negative correlation between the two parameters[13]. These suggest that the attenuation of hyperglycemia and hyperinsulinemia after ingestion of αAI-containing meals may affect plasma 3-OHBA levels. The aim of the present study was to examine 1) the effect of αAI on the acute plasma glucose response to ad libitum feeding, and 2) the effects of the decrease in plasma glucose on plasma 3-OHBA and insulin during chronic food restriction.

* Corresponding author.

Materials and methods

Alpha-AI preparation. The αAI used in this study was Deobesitogen® (BIOS Co. Ltd., Japan). The dry matter was analyzed, and was found to consist of 42.0% protein, 54.8% carbohydrate, and undetected phytohemagglutinin[14]. αAI activity was 6.4 U/mg as salivary type and 1.8 U/mg as pancreatic type.

Preparation of diet. High starch diet was prepared by mixing equal amounts of corn starch and standard powdered food (# CE-2; Clea Inc. Ltd., Japan). The diet contained 11.8% protein, 76.4% carbohydrate and 2.2% fat. For the αAI test diet, αAI was added to the high starch diet at 5% by weight.

Animals. Male Wistar King A rats, 280–350 g, were used. They were housed in a room illuminated daily from 08.00 to 20.00 h (a 12-h light-dark cycle) and maintained at 21 ± 2 °C with humidity at 55 ± 5%. The rats were allowed free access to tap water and standard

powdered food if not otherwise described. Prior to each experiment, all rats were handled for 5 min daily for 5 successive days to equilibrate their arousal levels.

Surgery. At least one week before testing, each rat was implanted with a right atrial silicone rubber catheter for blood sampling. After the rat was anesthetized with sodium pentobarbital (50 mg/kg i.p.), the catheter was inserted through the right external jugular vein with the inner end fixed just outside the right atrium. The outer end of the catheter was attached to an L-shaped stainless steel tube anchored to the skull. The catheter was filled with non-heparinized polyvinylpyrolidone (0.5 g/ml). Details of the procedure have been described elsewhere[15,16].

Measurement of plasma glucose. Matched on the basis of body weight, 10 rats were divided equally into 2 groups. Food was withheld from the rats for 24 h before feeding the high starch diet. Free access to water was maintained. Rats were fed 1 g of the high starch diet containing αAI (αAI group) or 1 g of the high starch diet without αAI (control group). The feeding of test diet was started at 13.00 h. The sample was completely consumed within 2 min. Blood samples were collected from the jugular vein through the atrial catheter under unrestrained and unanesthetized conditions[16]. The implanted sampling tube was attached to a Venoject Multi Sampling Needle (Terumo Co., Japan) to prevent air from entering the system. Sampling volume did not exceed 0.2 ml. Details of this blood sampling system were described previously[16]. Samples were taken 15 min before and at 15, 30 and 60 min after the start of feeding. One rat in the control group was excluded from the study because its catheter became blocked. Plasma glucose was measured by the glucose oxidase-paraaminophenol method[17]. The response area for plasma glucose[18] was calculated as the area under the plasma glucose response curve above the prefeeding plasma glucose level.

Measurement of plasma 3-OHBA and insulin. Matched on the basis of body weight, 10 rats were divided equally into 2 groups. After 3 days adaptation to the high starch diet ad libitum using a previously described feeding box[19], food intake was restricted to 10 g/day of the high starch αAI diet (αAI group) or the high starch diet without αAI (control group) for 7 successive days. Each day's sample diet was completely consumed. Blood sampling and measurement of body weight were carried out at 13.00 h before, and 1, 3, and 7 days after the start of food restriction. Sampling volume did not exceed 0.4 ml. One rat in the control group was excluded because of catheter occlusion. Plasma 3-OHBA was measured by the method of Williamson, Mellanby and Krebs with β-hydroxybutyric acid dehydrogenase[20]. Plasma insulin was measured using a double antibody solid phase radioimmunoassay[21].

Statistical analyses. Data are presented as mean ± SEM. To evaluate response of plasma glucose levels and changes in plasma 3-OHBA and insulin levels in each group, one-way analysis of variance was used. For multiple comparisons, Fisher's protected least significant difference test was performed. Two-sample *t* test was used to compare between groups.

Results

Response of plasma glucose to high starch diet. The response of plasma glucose to 1 g high starch diet with or without αAI following a 24-h fast is shown in figure 1. Plasma glucose in both groups increased in response to the high starch diet compared with corresponding initial values ($p < 0.01$ for each comparison vs the appropriate 0 min values, except $p < 0.05$ for control group at 60 min). The increase in glucose at 15 min was attenuated by αAI addition compared with the appropriate control value ($p < 0.05$). In the αAI group, plasma glucose level increased up to 30 min after feeding, while glucose levels in the control group peaked at 15 min, and then decreased. There was no significant difference in glucose levels between the two groups either at 30 min or 60 min after feeding. The response area for plasma glucose was not significantly different between the two groups (αAI group: 19.63 ± 1.84 mg · h/dl, control group: 19.34 ± 1.00 mg · h/dl).

Changes in plasma 3-OHBA, plasma insulin and body weight during food restriction. Changes in the time course of plasma 3-OHBA levels during the 7-day food-restriction period are shown in figure 2. Compared with the level before food restriction, 3-OHBA in both groups increased on 1 day after the start of food restriction ($p < 0.01$ for each). There was no significant difference between the two groups on 1 day after the start of restriction. The levels of 3-OHBA in both groups 3 days

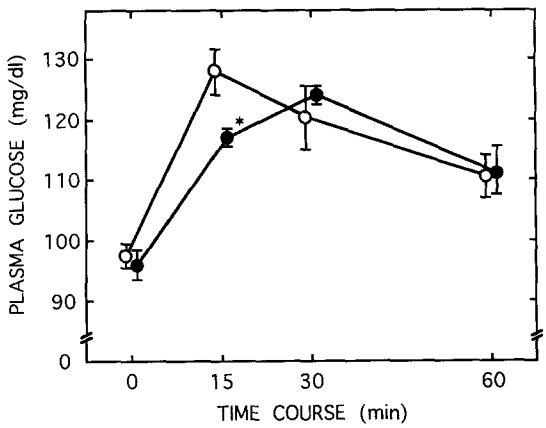

Figure 1. Response of plasma glucose to 1 g high starch diet following 24-h fasting: ●—●, high starch diet with α-amylase inhibitor (n = 5); ○—○, high starch diet without α-amylase inhibitor, as the control (n = 4). Each value, mean ± SEM. * = $p < 0.05$ vs the control.

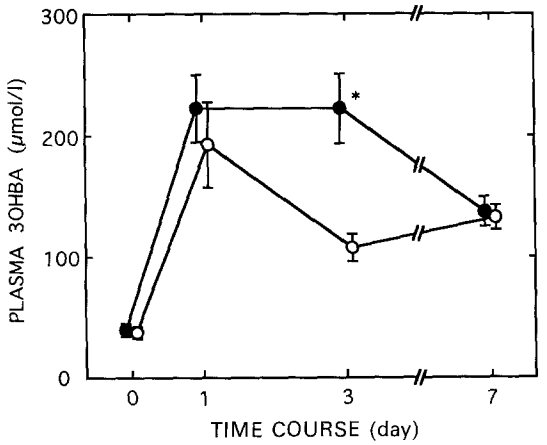

Figure 2. Changes in plasma 3-hydroxybutyric acid (3-OHBA) levels during the 7-day food-restriction periods: ●—●, high starch diet with α-amylase inhibitor (n = 5); ○—○, high starch diet without α-amylase inhibitor, as the control (n = 4). Each value, mean ± SEM. * = $p < 0.01$ vs the control.

after the start of restriction were still higher than the respective levels before restriction ($p < 0.01$ for αAI, $p < 0.05$ for control). However, the αAI group maintained its high level of 3-OHBA 3 days after restriction, while the control group showed significantly decreased levels compared with that after 1 day of food restriction ($p < 0.05$). There was a significant difference in the levels after 3 days of restriction between groups ($p < 0.01$). Plasma 3-OHBA levels in the αAI group 7 days after the start of restriction declined significantly compared with levels measured after 3 days of restriction ($p < 0.05$), such that 3-OHBA levels were similar to those in the control group. However, 3-OHBA levels 7 days after the start of restriction in both groups were still higher than their initial values ($p < 0.01$ for each). Changes in plasma insulin levels after 3 days of food restriction are shown in the table. In the αAI group, plasma insulin levels 3 days after food restriction decreased significantly compared with the corresponding levels in the control group ($p < 0.05$) or before restriction ($p < 0.05$). Differences in body weight reduction due to food restriction between these two groups were not significant (αAI group: before, 309 ± 10 g; 1 day, 298 ± 10 g; 3 days, 285 ± 8 g; 7 days, 263 ± 8 g; control group: before, 305 ± 9 g; 1 day, 295 ± 8 g; 3 days, 276 ± 7 g; 7 days, 258 ± 8 g).

Table. Changes in plasma insulin levels during the food-restriction periods.

	Plasma insulin (ng/ml)	
	before	3 days
αAI group (n = 5)	5.41 ± 0.99	3.11 ± 0.11[a,b]
control group (n = 4)	4.98 ± 0.44	4.39 ± 0.58

Each value, mean ± SEM. Before, before food restriction. 3 days, 3 days after food restriction. [a] $p < 0.05$ vs control group. [b] $p < 0.05$ vs the corresponding value before loading α-amylase inhibitor (αAI).

Discussion

Digestion of starch was delayed in αAI-treated rats without affecting total starch digestion[6]. The present study confirmed this observation: αAI produced a delayed hyperglycemic response under ad libitum feeding conditions, but it did not affect the response area for plasma glucose. In our preliminary experiments, αAI-containing meals administered to rats for 14 days produced no significant decrease in body weight (unpublished). This finding was confirmed in the present study: the body weight reduction in the αAI group during 7 days of food restriction was similar to that in the control group. Thus, retarded digestion and absorption of starch following αAI treatment delayed the hyperglycemic response, while leaving total energy intake unaffected.

Despite the lack of effect of αAI on energy intake, these findings raise the question of whether the delayed hyperglycemic response may be associated with changes in other metabolic responses. Accordingly, plasma levels of 3-OHBA were measured. When animals are starved or food-restricted, plasma 3-OHBA starts to increase, because the decrease in energy intake induces a shift in energy source from glucose to lipid[9]. This may explain why both groups fed with high starch diet showed a similar increase in 3-OHBA after 1 day of food restriction. The results suggest that the magnitude of energy deficiency was similar for both groups.

After 3 days of food restriction, the αAI group maintained 3-OHBA levels higher than the control. Since total calorie intake did not differ between groups, it is not likely that a difference in energy deficit can explain the difference in plasma levels of 3-OHBA between groups. The elevated level of 3-OHBA in the αAI group might be the result of decreased insulin secretion, due to the delayed hyperglycemic response reducing insulin secretion during chronic αAI loading. In fact, the present study confirmed that αAI reduced insulin secretion after 3 days of food restriction.

The higher maintenance of 3-OHBA resulting from lower insulin secretion in response to αAI treatment may be due to 1) increased 3-OHBA production and/or 2) decreased 3-OHBA utilization. A direct correlation has been shown between the rates of production and utilization and the blood concentration of ketone bodies[22]. Insulin decreases lipolysis and ketogenesis[13]. Thus, the αAI may increase both production and utilization of 3-OHBA.

In chronic starvation for more than 4 days, body fat stores in rats are almost exhausted, thus diminishing plasma levels of free fatty acids, glycerol and ketone bodies[9]. This may explain why there was no difference

in 3-OHBA levels between the control and αAI groups after 7 days of food restriction.

The present study demonstrates that αAI administration to rats feeding ad libitum does not change energy intake or body weight. However, the delayed hyperglycemic response and lower insulin level produced by αAI treatment may be useful to the patient with non-insulin dependent diabetes mellitus[23]. Attention should be drawn to the fact that changes in body weight do not necessarily reflect changes in body composition. The results showing that αAI elevates 3-OHBA in 3-day food restriction suggest that it may be useful in enhancing fat utilization as energy source, and hence sparing protein during food restriction as weight control.

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SCIENTIFIC CORRESPONDENCE

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