Intragastric Administration of Allyl Isothiocyanate Reduces Hyperglycemia in Intraperitoneal Glucose Tolerance Test (IPGTT) by Enhancing Blood Glucose Consumption in Mice

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Summary We investigated the effects of allyl isothiocyanate (AITC) on the blood glucose levels of mice using an intraperitoneal glucose tolerance test. The intragastric administration of 25 mg/kg body weight AITC reduced the increase in blood glucose level after 2 g/kg body weight glucose was given intraperitoneally, compared with that of control mice. To elucidate the mechanism responsible for the reduction, respiratory gas analysis employing 13C-labeled glucose was performed. The intragastrically administering AITC increased 13CO2 emission, compared to vehicle, after intraperitoneal administration of 13C-labeled glucose. This indicated that AITC increased the utilization of exogenously administered glucose, which was excessive glucose in the blood. To examine whether transient receptor potential (TRP) channels mediated this reduction in the blood glucose levels, we used TRPA1 and TRPV1 knockout (KO) mice. Intragastrically administering AITC reduced the increase in the blood glucose level in TRPA1 KO mice but not in TRPV1 KO mice. These findings suggest that dietary AITC might reduce the increases in blood glucose levels by increasing the utilization of excessive glucose in the blood by activating TRPV1.

Key Words AITC, TRPV1, glucose metabolism, respiratory gas analysis, mice

Controlling glucose metabolism is important to prevent the development of metabolic disorders such as obesity and type 2 diabetes. Recent studies revealed that postprandial dysmetabolism (i.e., hyperglycemia and hyperlipidemia) is related to the risk of developing obesity and cardiovascular disease (1–4). Postprandial hyperglycemia and hyperlipidemia induce endothelial dysfunction associated with increased oxidative stress and vascular inflammation, and are involved in the pathogenesis of atherosclerosis. Therefore, it is important to control postprandial metabolic states and reduce postprandial metabolic abnormalities.

The transient receptor potential (TRP) channel family is composed of a wide variety of cation-permeable channels and shows great diversity in its mechanisms of activation. TRPV1 and TRPA1 are cation channels belonging to the TRP channel family, and are activated by high (5, 6) and low (7, 8) noxious temperatures, respectively. Interestingly, they are also activated by spicy or pungent compounds in foods, such as capsaicin, piperine, cinnamaldehyde and allicin (5, 7, 9, 10). Recent studies indicate that the activation of TRPV1 or TRPA1 is involved not only in nociception and thermosensation but also thermoregulation and energy metabolisms (11–15).

Allyl isothiocyanate (AITC) is a natural compound in plants belonging to the family Cruciferae, and is the pungent ingredient in mustard, horseradish and wasabi. AITC activates TRPA1 (16), and recent studies suggest that it could activate not only TRPA1 but also TRPV1 (17, 18).

Research has suggested that TRPV1 is involved in the emergence of diabetes and obesity (19–22). Long-term treatment with TRPV1 antagonists reduces fasting glucose, triglyceride, and insulin levels in ob/ob mice (21). Moreover, dietary capsaicin, which is a TRPV1 agonist, is expected to improve not only obesity-induced inflammation but also obesity-related metabolic disorders such as insulin resistance (20). We previously demonstrated that intragastric administration of AITC increases carbohydrate oxidation via TRPV1 (18). Based on these facts, TRPV1 is considered to play a key role in glucose metabolism.

Approaches for suppressing postprandial hyperglycemia have been studied, but that research was generally aimed at preventing glucose absorption by inhibiting digestive enzymes (23–25). We considered that the increase in carbohydrate oxidation by administering
AITC might enhance glucose metabolism under excessively increased blood glucose conditions, such as during the postprandial period, followed by suppressing hyperglycemia. The approach for suppressing postprandial hyperglycemia by increasing the utilization of blood glucose has not been well studied, and it is expected to become a novel approach for suppressing postprandial hyperglycemia.

In the present study, we investigated the effects of intragastric administration of AITC on the blood glucose levels in mice by using an intraperitoneal glucose tolerance test (IPGTT). To elucidate the relationship between carbohydrate oxidation and blood glucose utilization, respiratory gas analysis of the changes in $^{13}$CO$_2$ emission after administering $^{13}$C-labeled glucose was performed. We also investigated the relationship between TRP channels and changes in the blood glucose levels by using TRPA1 knockout (KO) mice and TRPV1 KO mice.

**MATERIALS AND METHODS**

**Animals.** Male C57BL/6 mice (Japan SLC, Inc., Hamamatsu, Japan) were used. Mutant TRPV1-null mice and TRPA1-null mice were generously provided by Dr. D. Julius (University of California, San Francisco, CA). The procedure for generating mutant mice is reported in previous literature (26, 27). Mutant mice were backcrossed into the C57BL/6 genetic background. The mice were housed in a standard cage and maintained at 23±2°C under a 12:12-h light-dark cycle (lights on 0600–1800 h) with free access to a commercial standard laboratory chow (MF; Oriental Yeast Co., Ltd., Tokyo, Japan) and drinking water. All experimental protocols were approved by the Institutional Animal Care and Use Committee of Kyoto University and were in complete compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

**Materials.** Allyl isothiocyanate was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). $\alpha$-Glucose was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). $^{13}$C-labeled glucose was obtained from Isotec (Miamisburg, OH). For intragastric administration, AITC was diluted in saline containing 3% ethanol and 10% Tween 80.

**Intraperitoneal glucose tolerance test.** The mice (14–17 wk old) were deprived of food overnight (about 13 h; 2300–1200 h) and had free access to water. They were administered glucose (2 g/kg body weight) intraperitoneally and then AITC (25 mg/kg body weight) or the vehicle intragastrically. Blood glucose levels were measured before and after AITC or vehicle was administered. For blood glucose measurements, blood samples were obtained from the tail vein and analyzed using a Glucocard Diameter (Arkray, Kyoto, Japan).

**Respiratory gas analysis.** The mice (14–17 wk old) were kept individually in a chamber for 12 h to attain a constant respiratory exchange ratio. AITC or vehicle was administered and the expired air was then analyzed. The oxidation of total carbohydrate was computed on the basis of oxygen consumption ($V_o_2$) and carbon dioxide production ($V_{CO_2}$). Gas analysis was performed using an open-circuit metabolic gas analysis system connected directly to a mass spectrometer (model Arco2000; ArcoSystem, Chiba, Japan). The gas analysis system is described in detail elsewhere (28, 29).
Briefly, each metabolic chamber had a 72-cm² floor and was 6 cm in height. Room air was pumped through the chambers at a rate of 0.5 L/min. Expired air was dried in a cotton thin column and then directed to an O₂/CO₂ analyzer for mass spectrometry.

On the basis of the volume of CO₂ production per unit of time (L/min; V CO₂) and V O₂, total carbohydrate oxidation was calculated using the stoichiometric equations of Frayn (30) as follows:

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\text{total carbohydrate oxidation} = 4.55 \times \text{V O}_2 - 3.21 \times \text{V CO}_2.
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Oxidation of exogenous carbohydrate in the IPGTT was assessed on the basis of the relative abundance of 13 CO₂ (13 CO₂/12 CO₂ ratio) in the respiratory gas after intraperitoneal administration of 13 C-labeled glucose. A solution containing 10% glucose and 0.02 mol/L of 13 C-labeled glucose was administered intraperitoneally to the mice (0.01 mL/g of body mass) and then AITC or vehicle was intragastrically administered. The total amount of administered 13 C-labeled glucose was 0.2 mmol/kg of body mass.

Data analysis. All values are presented as means±SE. The effects of intragastrically administering AITC on blood glucose levels, the relative abundance of 13 CO₂, cumulative carbohydrate oxidation and V O₂ were examined by two-way repeated-measures ANOVA (Prism 5.0; GraphPad Software, San Diego, CA) followed by an unpaired t-test (see Figs. 1, 3, and 5). The effects of intragastric administration of AITC on the area under the curve (AUC) of blood glucose and average the relative abundance of 13 CO₂ and cumulative carbohydrate oxidation and V O₂ for 2 h (see Figs. 2, 4, and 6) were examined by using an unpaired t-test.

**RESULTS**

**Effects of intragastric administration of AITC on blood glucose levels**

The blood glucose levels after intragastric administration of AITC in the IPGTT were measured. The dose of AITC was determined from our previous study, wherein it was considered to be sufficient to affect energy metabolism (18). The blood glucose levels increased after intraperitoneal glucose administration; however, intragastric administration of AITC reduced the increase in blood glucose level compared with vehicle administration for 15–60 min after administration (Fig. 1A). The AUC for the blood glucose, 2 h after administration, was lower in the AITC-treated group than in the vehicle-treated group (Fig. 2A).

**Effects of intragastric administration of AITC on excess blood glucose utilization**

We considered that the increase in carbohydrate oxidation by administering AITC might have increased the utilization of excessive glucose in the blood, followed by a reduction in the increase in blood glucose level as seen by the IPGTT. To elucidate the relationship between the increase in carbohydrate oxidation and the reduction in the increase in blood glucose level, we measured the 13 CO₂/12 CO₂ ratio in respiratory gas after intragastric administration of AITC with 13 C-labeled glucose. In the IPGTT, an increase in blood glucose levels was derived from intraperitoneally administered glucose. Therefore, an increase in the utilization of intraperitoneally administered glucose means an increase in the utilization of excessive blood glucose.
Intragastric administration of AITC elevated the $^{13}$CO$_2$/$^{12}$CO$_2$ ratio for 10–50 min after the administration, compared to vehicle administration (Fig. 1B). AITC increased carbohydrate oxidation for 20–110 min after the administration, compared with vehicle (Fig. 1C).

The average of the $^{13}$CO$_2$/$^{12}$CO$_2$ ratio and the cumulative total carbohydrate oxidation for 2 h after administration were higher in the AITC-treated group than in the vehicle-treated group (Fig. 2B and C). $V_O_2$ was not influenced for at least 2 h after administration (Figs. 1D).
Contribution of TRPA1 in enhancing the utilization of excessive blood glucose

To examine the contribution of TRPA1 in reducing the increase in blood glucose level by administering AITC, TRPA1 KO mice were employed. In TRPA1 KO mice, intragastric administration of AITC reduced the increase in blood glucose level in the IPGTT compared with the vehicle administration for 15–60 min after administration (Fig. 3A). This finding was similar to

Fig. 5. (A) Changes in the blood glucose levels of TRPV1 KO mice administered AITC or vehicle (control) in IPGTT. Values are expressed as means ± SE, n=11; *p<0.05 (two-way repeated-measures ANOVA, followed by an unpaired t-test). (B–D) Changes in the relative abundance of $^{13}$CO$_2$, carbohydrate oxidation and $V_O_2$ of TRPV1 KO mice administered AITC or vehicle (control) in IPGTT. Values are expressed as means ± SE (n=11). There is no significant difference between the groups.

Fig. 6. (A) AUC of blood glucose levels of TRPV1 KO mice in IPGTT. Values are expressed as means ± SE (n=11). (B–D) Average relative abundance of $^{13}$CO$_2$, cumulative carbohydrate oxidation and $V_O_2$ of TRPV1 KO mice administered AITC or vehicle (control) in IPGTT for 2 h. Values are expressed as means ± SE (n=11). There is no significant difference between the groups.

and 2D).

Contribution of TRPA1 in enhancing the utilization of excessive blood glucose

To examine the contribution of TRPA1 in reducing the increase in blood glucose level by administering AITC, TRPA1 KO mice were employed. In TRPA1 KO mice, intragastric administration of AITC reduced the increase in blood glucose level in the IPGTT compared with the vehicle administration for 15–60 min after administration (Fig. 3A). This finding was similar to
that observed in wild type (WT) mice. The AUC for the blood glucose, 2 h after administration, was lower in the AITC-treated group than in the vehicle-treated group (Fig. 4A).

In the respiratory gas analysis, intragastric administration of AITC elevated the $^{13}$CO$_2$-$^{12}$CO$_2$ ratio at 20–30 and 60 min after the administration, compared to vehicle administration (Fig. 3B). AITC increased carbohydrate oxidation for 20–60 min after the administration compared with vehicle (Fig. 3C). The average of the $^{13}$CO$_2$-$^{12}$CO$_2$ ratio and the cumulative total carbohydrate oxidation for 2 h after administration were higher in the AITC-treated group than in the vehicle-treated group (Fig. 4B and C). $V_O_2$ was not influenced for at least 2 h after administration (Figs. 3D and 4D).

**Contribution of TRPV1 in the enhancement of utilization of excessive glucose in blood**

We previously reported the involvement of TRPV1 in the increase in carbohydrate oxidation by AITC (18). To examine the contribution of TRPV1 in reducing the increase in blood glucose level by administering AITC, IPGTT was performed on TRPV1 KO mice. Intragastric administration of AITC did not reduce the increase in blood glucose level caused by the IPGTT. This finding differed from that observed for WT mice (Fig. 5A). One hundred twenty minutes after administration, the blood glucose levels of the AITC-treated group was higher than those of the vehicle-treated group. However, there was no significant difference between the groups in terms of the AUC for the blood glucose 2 h after administration (Fig. 5B).

Using respiratory gas analysis, we found that there was no significant difference between groups in the $^{13}$CO$_2$-$^{12}$CO$_2$ ratio (Fig. 5B). There was no significant difference between groups in the average of the $^{13}$CO$_2$-$^{12}$CO$_2$ ratio for 2 h after administration (Fig. 6B). These findings differed from those observed in WT mice. AITC slightly increased carbohydrate oxidation for 2 h after administration compared to vehicle administration (Fig. 5C); however, there was no significant difference between groups in the cumulative total carbohydrate oxidation for 2 h after administration (Fig. 6C). $V_O_2$ was not influenced for at least 2 h after administration (Figs. 5D and 6D).

**DISCUSSION**

In the present study, we observed that intragastric administration of AITC reduced the increase in blood glucose level for 15–60 min after administration in the IPGTT. Respiratory gas analysis showed that intragastric administration of AITC increased the $^{13}$CO$_2$-$^{12}$CO$_2$ ratio for 10–50 min after administration. This period was in accordance with the period of the reduction in the increase in blood glucose level by AITC. The increase in the $^{13}$CO$_2$-$^{12}$CO$_2$ ratio meant an increase in the oxidation of intraperitoneally administered $^{13}$C-labelled glucose. The increase in blood glucose level was because of the intraperitoneally administered $^{13}$C-labelled glucose. Therefore, these results indicate that intragastric administration of AITC reduces the increase in blood glucose level by increasing the utilization of excessive glucose in the blood.

AITC is a typical TRPA1 agonist (7, 16). In TRPA1 KO mice, however, intragastric administration of AITC reduced the increase in blood glucose level and increased the $^{13}$CO$_2$-$^{12}$CO$_2$ ratio in the IPGTT, which is similar to that observed in WT mice. These results agree with our previous results that TRPA1 is not involved in the increase in carbohydrate oxidation by administering AITC (18). Therefore, TRPA1 is considered not to be involved in reducing the increase in blood glucose level by intragastrically administering AITC.

In TRPV1 KO mice, intragastric administration of AITC did not reduce the increase in blood glucose level and did not increase the $^{13}$CO$_2$-$^{12}$CO$_2$ ratio in the IPGTT. Therefore, TRPV1 is involved in reducing the increase in blood glucose level by intragastrically administering AITC.

The blood glucose levels at 2 h after administering AITC were higher than those of the control in WT mice. This phenomenon was also observed in TRPV1 KO mice but not in TRPA1 KO mice. Therefore, this elevation in blood glucose level was involved not in TRPV1 but in TRPA1. AITC might not only bring about a reduction in the increase in blood glucose level, which is involved in TRPV1, but also cause side effects such as increasing the blood glucose levels for 2 h after administration, which is involved in TRPA1. Further studies are needed to elucidate the mechanism about increased blood glucose levels.

The mechanisms by which AITC increases carbohydrate oxidation remain unclear. Previous studies reported that administering AITC induces adrenaline secretion (11) and that capsaicin, which is a typical TRPV1 agonist, induces adrenaline secretion primarily by activating the adrenal sympathetic nerve (14). Therefore, it was assumed that adrenaline secretion through the activation of the central nervous system was induced by AITC and that glucose uptake was increased via adrenoreceptor activation by adrenaline or noradrenaline in skeletal muscles and adipose tissues. It remains unclear where and how glucose was metabolized, for which further studies are required.

Recent studies suggested that TRPV1 KO mice have insulin resistance or obesity (19–22). Therefore, it is possible that impairments in glucose metabolism, like insulin resistance, cause differences in blood glucose level between WT mice and TRPV1 KO mice. However, the changes in blood glucose level of the vehicle-treated group in TRPV1 KO mice were similar to those in WT mice, which indicates that there was no difference in glucose tolerance ability between WT mice and TRPV1 KO mice. Therefore, we believe that the differences in blood glucose level between WT mice and TRPV1 KO mice were not derived from chronic changes in glucose metabolism between mice types and that TRPV1 might play a key role in the control of glucose metabolism.

Our results showed that intragastric administration of AITC reduced the increase in blood glucose level after glucose loading by increasing the utilization of excessive glucose.
blood glucose. The approach for suppressing postprandial hyperglycemia, which entails increasing the utilization of blood glucose, is expected to become a novel approach for suppressing postprandial hyperglycemia. However, it remains unclear how AITC reduced the increase in blood glucose level, for which further studies are required to consider applying this discovery in humans.

In conclusion, we demonstrated that intragastric administration of AITC reduced the increase in blood glucose level in the IPGTT and that the reduction in the increase was derived from the increased utilization of excessive blood glucose. We also showed that these effects were involved in not TRPA1 but TRPV1 and suggested that the activation of TRPV1 may be involved in controlling the blood glucose levels.

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