1-Tryptophan Suppresses Rise in Blood Glucose and Preserves Insulin Secretion in Type-2 Diabetes Mellitus Rats

Tomoko INUBUSHI1, Norio KAMEMURA2, Masataka ODA3, Jun SAKURAI1, Yutaka NAKAYA4, Nagakatsu HARADA4, Midori SUIZAGA1, Yoichi MATSUMAGA4,* Kazumi ISIDOH2 and Nobuhiko KATUNUMA2

1Faculty of Life Science, 2Institute for Health Sciences, and 3Faculty of Pharmaceutical Sciences, Tokushima Bunri University, 180 Nishihamabayuji, Yamashiro-cho, Tokushima, Tokushima 770–8514, Japan
4Department of Nutrition and Metabolism, School of Medicine, The University of Tokushima, 3–18–15 Kuramoto-cho, Tokushima, Tokushima 770–8503, Japan
(Received May 24, 2012)

Summary Ample evidence indicates that a high-protein/low-carbohydrate diet increases glucose energy expenditure and is beneficial in patients with type-2 diabetes mellitus (T2DM). The present study was designed to investigate the effects of 1-tryptophan in T2DM. Blood glucose was measured by the glucose dehydrogenase assay and serum insulin was measured with ELISA in both normal and hereditary T2DM rats after oral glucose administration with or without 1-tryptophan and tryptamine. The effect of tryptophan on glucose absorption was examined in the small intestine of rats using the everted-sac method. Glucose incorporation in adipocytes was assayed with [3H]-2-deoxy-D-glucose using a liquid scintillation counter. Indirect computer-regulated respiratory gas-assay calorimetry was applied to assay energy expenditure in rats. 1-Tryptophan suppressed both serum glucose and insulin levels after oral glucose administration and inhibited glucose absorption from the intestine. Tryptamine, but not 1-tryptophan, enhanced insulin-stimulated [3H]-glucose incorporation into differentiated adipocytes. 1-Tryptophan increased glucose-associated energy expenditure in rats in vivo. 1-Tryptophan-rich chow consumed from a young age preserved the secretion of insulin and delayed the progression of T2DM in hereditary diabetic rats. The results suggested that 1-tryptophan suppresses the elevation of blood glucose and lessens the burden associated with insulin secretion from β-cells.

Key Words 1-tryptophan, OGTT, tryptamine, type-2 diabetes mellitus

The main mode of action of currently available medications for type-2 diabetes mellitus (T2DM) is stimulation of insulin secretion from pancreatic β-cells (1). However, such glucose-lowering agents could burden β-cells and worsen prognosis. Regular muscle exercise enhances insulin sensitivity and consequently reduces the burden on β-cells. Clinical evidence indicates that a high-protein and low-carbohydrate diet is beneficial in the treatment of T2DM (2–4).

Although the high protein in the diet reduced blood glucose levels, resulting in the reduction of the burden of pancreatic β-cells, its constituent amino acids have various effects on blood glucose level (5); it was reported that amino acid combinations such as isoleucine and serine increased insulin sensitivity (6), whereas L-glutamine, glycine and threonine decreased maximal insulin responsiveness of the glucose transport system in isolated adipocytes (7). The effects of 1-tryptophan on blood glucose level have been complex, because not only 1-tryptophan itself upregulates gluconeogenesis in liver (8), but also its metabolites including 5-hydroxy-tryptamine and serotonin (5-HT) modulate insulin secretion (9) and glucose uptake into skeletal muscle (10), as well as melatonin-stimulated insulin secretion (11). Recently, it was reported that oral administration of 1-tryptophan for 15 d did not modify glyceremia or insulinemia, but raised melatonin in T2DM model rats (12).

In the present study, we report two novel effects for 1-tryptophan: 1-tryptophan itself decreases glucose absorption from the small intestine, and its metabolite, tryptamine, increases glucose uptake into adipocytes. Finally, 1-tryptophan-rich-chow consumed from a young age effectively prevents exhaustion of β-cells in old T2DM rats. We speculate that 1-tryptophan and its metabolites play an important role in “the specific dynamic action” and that 1-tryptophan is potentially useful therapeutically in T2DM.

EXPERIMENTS

Experimental animals. Four-week-old male normal Sprague-Dawley (SD) rats from Japan SLC, Inc. and hereditary type-2 diabetes Sprague-Dawley Torii rats (SDT) from CLEA Japan, Inc., which develop spontaneous T2DM, were used in this study. All rats were fasted at 17:00 for 16-h before the experiments. SDT rats were
Inubushi T et al.

**Fed normal chow** (MF, Oriental Yeast Co., Ltd.), and L-tryptophan-rich-chow (add 37.5 mg L-tryptophan to 100 g chow, total 280 mg of L-tryptophan in 100 g of normal-chow and 317.5 mg of that in 100 g of L-tryptophan-rich-chow). The rats were allowed food ad libitum. All animal experiments described in this study were approved by the Animal Review Committee of Tokushima Bunri University before the study.

**Oral glucose tolerance test (OGTT) and insulin assay.**
The OGTT was performed after 16-h fasting in SD or SDT rats with glucose (2 g/kg BW) alone (n=8), plus L-tryptophan (62.5 mg/kg BW) orally (n=6) or intraperitoneally (n=7), or plus D-tryptophan (62.5 mg/kg BW) orally (n=5). Blood sugar level (A) and serum insulin level (B). Data in (A) and (B) are mean±SD. *p<0.05, by Wilcoxon-Mann-Whitney’s test. Glucose uptake in everted-sac (C). Everted-small intestine isolated from 8-wk-old rat. Sac containing 1 mL of saline was incubated in 10 g/dL of glucose solution added L-tryptophan (0.1–0.3 g/dL) or D-tryptophan (0.3 g/dL) at 37˚C. Glucose concentration in the saline was assayed at 30 min and 60 min. Data are mean±SD. *p<0.05, and **p<0.01 by ANOVA.

**Assay of glucose absorption using an everted-sac of rat small intestine in vitro.** The method of Wilson and Wiseman was used for measurement of glucose absorption (14). A 20-cm-long portion of the small intestine was isolated from 8- or 9-wk-old SD rats and one end of the small intestine was bound to form a sac, and the sac was everted so that the serosal layer was inside and the mucosal layer was outside. The sac was filled with 1 mL of saline and incubated in glucose solution (10 g/dL) at 37˚C under aeration. The saline samples (120 µL) were pumped out from the inside of the sac for 30 and 60 min, and glucose concentrations in saline were assayed using the Somogyi-Nelson method (15, 16).

**Preparation of differentiated adipocytes and glucose uptake assay.** Preadipocytes 3T3-L1 were obtained from the Health Science Research Resources Bank, Japan (17). They were inoculated at a concentration of 1.0×10^4 cells/mL in 24 well gelatin coated plates (IWAKI, Japan) and cultured for 5-d. The cells were allowed to differentiate in high-glucose Dulbecco’s modified Eagle medium (DMEM) containing 1 µM dexamethasone, 10 µg/mL insulin, 0.5 mM 3-isobutyl-1-methyl-xanthine and 10% fetal bovine serum (FBS) for another 2-d. The culture medium were replaced with high-glucose DMEM containing 10 µg/mL insulin and 10% FBS, and cultured
for a further 10-d. Cell differentiation was confirmed by microscopic examination after Oil Red O staining ( Supplementary Fig. 1) and these cells were used as the differentiated adipocytes. The differentiated adipocytes were incubated in insulin-free high-glucose DMEM without FBS for 4-h and washed 3 times with 5 mM potassium dihydrogen phosphate, 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (pH 7.4), 1 mM MgSO$_4$. 1 mM CaCl$_2$, 136 mM NaCl, and 4.7 mM KCl designated as Kreb’s Ringer phosphate HEPES (KRPH) buffer (18). The cells were incubated with 0–100 μM L-tryptophan or tryptamine in the presence or absence of 10 μg/mL insulin in KRPH buffer at 37°C for 20 min. After the addition of [3H]-2-deoxy-D-glucose (75 kBq/mL, Perkin-Elmer, Walton, MA) and 2-deoxy-D-glucose (100 μM), the cells were incubated at room temperature for 20 min. After washing 3 times with KRPH buffer, the culture medium was replaced with 0.1 n NaOH at 0.5 mL/well for 16-h and the lysates (400 μL) were neutralized with 1 M HCl. The samples were added to Clearsol® scintillation cocktail (Nacalai Tesque, Inc., Kyoto, Japan) and incorporated [3H]-2-deoxy-D-glucose was measured by a liquid scintillation counter LSC-6100 (Hitachi-Aloka Medica, Tokyo, Japan).

Assay of energy expenditure in rats. An indirect computer-regulated respiration gas-assay calorimeter (Oxymax®, Columbus Instruments, Columbus, OH) was used to assay the energy expenditure in rats. The respiratory gas was circulated at 2.0 L/min. Either glucose (2 g/kg BW) or glucose plus l-tryptophan (62.5 mg/kg BW) was administrated orally.

Statistical analysis. Data are presented as mean± standard deviation. Differences in blood glucose levels between animal groups were analyzed by the Wilcoxon-Mann-Whitney test using Kaleidograph (Ver. 3.6, Synergy Co., PA). Group data were compared by one-way analysis of variance (ANOVA). A p value <0.05 (asterisk) or <0.01 (double asterisks) denoted the presence of a statistically significant difference. All statistical analyses were performed using The Statistical Package for Social Sciences (Ver 13.0, SPSS, Tokyo, Japan).

RESULTS

Per-oral-administrated l-tryptophan suppresses hyperglycemia with glucose load

L-Tryptophan was co-administrated orally in combination with glucose, and blood glucose levels were assayed using tail blood samples every 30 min. L-Tryptophan administration both via oral and intraperitoneal routes suppressed the increase in blood glucose level to levels similar to those after glucose load at 30 and 60 min (Fig. 1A). Interestingly, the suppression by d-tryptophan was lower than that by L-tryptophan at all time periods of the OGTT.

L-Tryptophan suppresses secretion of insulin after glucose load

Insulin levels was measured before and at 30, 40, and 60 min after oral administration of glucose plus l-tryptophan. L-Tryptophan significantly suppressed the insulin levels after glucose load at both 30 and 40 min (Fig. 1B).

L-Tryptophan inhibits glucose-absorption from small intestine

The amount of glucose absorbed from the small intestine was quantified directly in the in vitro system of the everted small intestine. Glucose added to the mucosal side was transported to the serosal side. L-Tryptophan, but not d-tryptophan, inhibited the transport of glucose in a dose-dependent manner (Fig. 1C).

Tryptamine enhances blood glucose incorporation into adipocytes

L-Tryptophan, but not d-tryptophan, reduced blood glucose level in OGTT (Fig. 1A). To elucidate the mechanisms of the effect of l-tryptophan, we analyzed insulin-stimulated glucose incorporation into differentiated adipocytes in the presence or absence of either l-tryptophan or its metabolite, tryptamine. Tryptamine, but not l-tryptophan, enhanced insulin-stimulated [3H]-glucose incorporation into the cells in a dose-dependent manner (Fig. 2A and B). Neither d-tryptophan nor 5-HT affected insulin-dependent 2-deoxy-D-glucose uptake (Supplementary Fig. 2A and B).

Indirect respiratory calorimetry revealed that rats fed
Inubushi T et al. with glucose plus L-tryptophan increased their energy expenditures. When L-tryptophan was co-administered with glucose, the energy expenditure increased significantly without any exercise compared to glucose only after 30 min of administration (Fig. 3).

Therapeutic effect of L-tryptophan-rich chow in hereditary type-2 diabetes rats

Next, we used L-tryptophan-rich chow in SDT rats to address the effect of L-tryptophan-rich chow on the onset and progression of T2DM. At first, we planned to add 18.75 mg of L-tryptophan to the normal chow (total 298.75 mg/100 g chow, designated as tryptophan-moderate-chow), and to feed SDT rats tryptophan-moderate-chow from 5 wk to 18 wk of age. The results of OGTT at various ages of SDT rats with tryptophan-moderate-chow did not show statistical difference from those with the normal chow. Thus, we used tryptophan-rich (317.5 mg tryptophan/100 g chow) chow for breeding SDT rats.

The mean fasting glucose level in 5-wk-old SDT rats (52.2 ± 5.2 mg/dL) was similar to that of the control

Fig. 3. L-Tryptophan modulates energy expenditure.

Energy expenditure of 5-wk-old SD rats fed glucose (2 g/kg BW) with or without L-tryptophan (62.5 mg/kg BW, each n = 5) was measured by an indirect computer-regulated calorimeter. Data are mean ± SD. *p < 0.05 by ANOVA.

Fig. 4. Glucose tolerance test in SDT rats. SDT rats were bred on normal chow (tryptophan (-), 280 mg/100 g chow, n=7) and tryptophan-rich chow (tryptophan (+): 375 mg of L-tryptophan was added to 1 kg chow, total 317.5 mg/100 g chow, n=6). They were subjected to OGTT at 5 wk of age (A), 12 wk of age (B), and 18 wk of age (C and D). Blood glucose (A–C) and insulin levels (D) were measured. Data are mean ± SD. *p < 0.05 by Wilcoxon-Mann-Whitney’s test.
Beneficial Effects of L-Tryptophan in Diabetes

A glucose tolerance test in SDT rats on normal chow showed increased glucose levels to 177 ± 33.2 mg/dL at 30 min, reaching a peak level of 220 ± 26.7 mg/dL at 60 min, and 128 ± 24.0 mg/dL later at 120 min. These were comparable to the value at 30 min but significantly higher than those at 60 and 90 min after administration in the normal SD rats. These results suggested that the potential insulin secretion level in SDT rats was lower than in SD rats at 5 wk of age. No differences were observed between the levels in SDT rats on L-tryptophan-rich chow and those on normal chow. At 12 wk of age, the fasting glucose levels in SDT rats with or without L-tryptophan-rich chow were 88 ± 6.89 and 91 ± 9.1 mg/dL, respectively (Fig. 4B). In the glucose-tolerance test, blood glucose levels in SDT rats on normal chow increased to 346 ± 35.1 mg/dL at 30 min, reached a peak level of 425 ± 58.3 mg/dL at 60 min and decreased to 269 ± 92.8 mg/dL at 120 min, whereas the values in SDT rats on L-tryptophan-rich chow were 282 ± 38.3, 356 ± 36.2, and 187 ± 66.6 mg/dL at 30, 60, and 120 min, respectively. The differences between blood glucose levels at 30 and 60 min were significant. At 18 wk of age, the fasting blood glucose levels in SDT rats with or without L-tryptophan-rich chow were increased (94 ± 9.1 and 117 ± 21.1 mg/dL, respectively), although the difference between the two groups was not significant (Fig. 4C). In the glucose-tolerance test, the blood glucose levels in SDT rats on normal chow increased to 347 ± 130.0 mg/dL at 30 min, reached a peak level of 517 ± 118.0 mg/dL at 60 min, and then decreased to 432 ± 114.6 mg/dL at 120 min, while in rats on L-tryptophan-rich chow these levels were 217 ± 73.4 mg/dL at 30 min, 354 ± 100.7 mg/dL at 60 min, and 253 ± 90.2 mg/dL at 120 min. The differences between 60 and 120 min were significant. These results suggest possible diminished insulin secretion in SDT rats on normal chow. To confirm this conclusion, we measured insulin contents in the blood of rats with or without L-tryptophan-rich chow (Fig. 4D). The basal insulin level in SDT rats fed normal chow was comparable to that in rats fed L-tryptophan-rich chow. Blood insulin level at 30 min after glucose administration in the former was lower than that in the latter, though blood glucose level in the former was higher than that in the latter. Thus, L-tryptophan-rich chow consumed from a young age effectively prevented exhaustion of β-cells in old-SDT rats, as well as the uptake of glucose from the small intestine in young and aged SDT rats.

Before differentiation

After differentiation

(Oil Red O staining)

Supplementary Fig. 1. Preadipocytes 3T3-L1 (A) and differentiated adipocytes (B) stained by Oil Red O. The differentiated adipocytes were used in the glucose uptake study.
Discussion

Based on the results of the present study, we propose two physiological functions for L-tryptophan: inhibition of glucose absorption from the small intestine and stimulation of blood glucose incorporation into cells. Figure 5 shows a schematic model of the effects of L-tryptophan on the absorption of orally administered glucose, including its metabolite, tryptamine, which stimulates blood glucose incorporation into the cell.

Several physiological effects have been reported for L-tryptophan, mainly in the veterinary medicine field (e.g., horses, oxen or sheep) with regard to its nutritional value, such as its effects on physical abilities (19). The present study demonstrated that co-administration of L-tryptophan with glucose increased the energy expenditure without any exercise (Fig. 3). The results suggest that L-tryptophan is an amino acid responsible, at least in part, for the “specific dynamic action” associated with a high-protein diet (20).

Oral administration of L-tryptophan was more effective than intraperitoneal injection with regard to the control blood glucose levels (Fig. 1A). This is based on the physiological functions of L-tryptophan, which inhibits glucose absorption from the small intestine. We tested the interaction of the purified porcine sodium glucose transporter (SGLT)-1 (21) and either D-glucose, L-tryptophan or D-tryptophan using the BIA Core 3000 system. Our preliminary results showed that both D-glucose and L-tryptophan interacted with SGLT-1 stronger than D-tryptophan did (see Supplementary Fig. 3). Unfortunately, we failed to obtain the results of competition analysis between D-glucose and L-tryptophan on SGLT-1 because of the small amount of SGLT-1 conjugated on the sensor chip. Further analysis of the competition assay between D-glucose and L-tryptophan should be done using a larger amount of SGLT-1 conjugated on the sensor chip.

In addition, the results showed that tryptamine, but not 5-HT, stimulated the rate of glucose uptake into adipocytes (Fig. 2B and Supplementary Fig. 2). Tryptamine is rapidly synthesized from L-tryptophan with the activity of aromatic amino-acid decarboxylase (22). Accordingly, the effects of L-tryptophan are thought to
be mediated by locally-produced tryptamine. Biochemically, 1% of L-tryptophan converts to tryptamine (23). As shown in Fig. 2B, 10 μM tryptamine significantly stimulated insulin-stimulated glucose uptake into differentiated adipocytes. Under physiological conditions, the blood l-tryptophan level is controlled at approximately 55 μM (24). Thus, an L-tryptophan-rich diet is necessary to achieve effective concentrations of tryptamine. Tryptamine also modulated insulin secretion, resulting in hypoglycemia in normal mice but not in T2DM mice (25). The effect of tryptamine was inhibited by the 5-HT receptor antagonist (26). The ratio of intracellular and extracellular 5-HT concentrations of β-cells controlled insulin secretion (10). Thus, the effect was possibly accomplished by intracellular 5-HT converted from tryptamine.

Experimental evidence suggests that L-tryptophan deficiency modulates glucose tolerance (8). Tryptophan enhanced glucose-mediated insulinotropic polypeptide (GIP) secretion in tryptophan-deficient or tryptophan-adequate animals (27). However, these reports did not focus on functions of monoamine, which is closely related to glucose metabolism (22). Oral administration of tryptophan raised melatonin levels in T2DM model rats (12). Melatonin improved insulin action and β-cell function (11). 5-HT increased uptake of glucose into the skeletal muscle and liver (9, 28), and then glycogen synthesis through cyclin-dependent-kinase-5 activation (29). Brain 5-HT content peaked at 1-h after refeeding whereas brain L-tryptophan content increased until 2-h and decreased after 4-h (30). This fact suggested brain monoamine profiles were possibly different from those of L-tryptophan in brain and monoamine in peripheral organs. Further analyses of the roles of brain and peripheral monoamines in glucose metabolism need to be carried out.

L-Tryptophan is also the precursor of NAD⁺ in the liver (22). Although the ratio of NADH/NAD⁺ is important for glucose metabolism (31), the amount of NADH itself also regulated them, because GAPDH activity was inhibited by NADH attachment (32). Thus, de novo NAD⁺ synthesis from L-tryptophan possibly participated in upregulation of glycogen synthesis (29).

The blood glucose level was controlled by not only glucose intake but also gluconeogenesis, which set the fasting blood glucose level (2). It was reported that L-tryptophan inhibited hepatic gluconeogenesis at the level of phosphoenolpyruvate formation (33). An in vivo study showed a paradoxical effect of L-tryptophan on the activity of phosphoenolpyruvate carboxykinase, a key enzyme for phosphoenolpyruvate synthesis (34). This effect on the gluconeogenesis of L-tryptophan is active in the normal rats but not in T2DM rats (35). The effects of L-tryptophan and its metabolites on in vivo gluconeogenesis should be further analyzed.

The current therapies for T2DM are mainly designed to stimulate insulin secretion from pancreatic β-cells (36). However, resting pancreas β-cells is a new paradigm in the treatment of T2DM (37) based on lessening the burden on β-cell function. The above studies add support to the inclusion of L-tryptophan in the treatment of T2DM. The results of glucose tolerance tests in SDT rats of various ages showed L-tryptophan-rich chow prevented the rise and kept the decrease of blood glucose level after administration (Fig. 4). Furthermore, the possible serum insulin level after administration was higher in SDT rats fed L-tryptophan-rich chow than in rats fed normal chow. The results suggest that the addition of L-tryptophan to food is a potentially effective treatment in these experimental diabetic rats. Hundley et al. (38) reported that administration of tryptophan increased blood glucose levels in fasting diabetic rats, which opposed to the effect seen in our study. The difference between the two studies could be due to differences in the study design; in our study, L-tryptophan-rich food was provided before the development of T2DM. Considered together, the above results and our findings suggest that the L-tryptophan is useful in delaying the progression of T2DM.

In conclusion, the present study demonstrated that L-tryptophan suppresses the rapid rise in blood glucose after a glucose-rich meal and lessens the burden of insulin secretion from β-cells. These results suggest that L-tryptophan is potentially beneficial in patients with T2DM. Further studies are needed to confirm the beneficial effects of L-tryptophan or its derivatives for dietary supplementation in patients with T2DM.

Conflict of interest: The authors declare no conflict of interest.

REFERENCES


8) Wittman JS 3rd. 1976. Alteration of glucose tolerance...


