Miglitol prevents diet-induced obesity by stimulating brown adipose tissue and energy expenditure independent of preventing the digestion of carbohydrates

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Abstract. Miglitol is an alpha-glucosidase inhibitor that improves post-prandial hyperglycemia, and it is the only drug in its class that enters the bloodstream. Anecdotally, miglitol lowers patient body weight more effectively than other alpha-glucosidase inhibitors, but the precise mechanism has not been addressed. Therefore, we analyzed the anti-obesity effects of miglitol in mice and in the HB2 brown adipocyte cell line. Miglitol prevented diet-induced obesity by stimulating energy expenditure without affecting food intake in mice. Long-term miglitol treatment dose-dependently prevented diet-induced obesity and induced mitochondrial gene expression in brown adipose tissue. The anti-obesity effect was independent of preventing carbohydrate digestion in the gastrointestinal tract. Miglitol effectively stimulated energy expenditure in mice fed a high-fat high-monocarbohydrate diet, and intraperitoneal injection of miglitol was sufficient to stimulate energy expenditure in mice. Acarbose, which is a non-absorbable alpha glucosidase inhibitor, also prevented diet-induced obesity, but through a different mechanism: it did not stimulate energy expenditure, but caused indigestion, leading to less energy absorption. Miglitol promoted adrenergic signaling in brown adipocytes in vitro. These data indicate that circulating miglitol stimulates brown adipose tissue and increases energy expenditure, thereby preventing diet-induced obesity. Further optimizing miglitol’s effect on brown adipose tissue could lead to a novel anti-obesity drug.

Key words: Adrenergic signaling, Alpha-glucosidase inhibitor, Brown adipose tissue, Diet-induced obesity, Energy expenditure

TYPE 2 DIABETES MELLITUS (T2DM) has become a major health problem around the world and is associated with high morbidity and excess mortality. Excessive weight is an established risk factor for T2DM, and the global epidemic of obesity largely explains the worldwide T2DM pandemic in the last 20 years [1-3]. Glucose-lowering medications, such as sulfonylureas, thiazolidinediones, and insulin, are useful for achieving glycemic control, but can make weight control challenging [4]. The history of anti-obesity drug development is tainted with multiple occasions of problems with side effects and subsequent market withdrawal, leading to a low rate of drug approval [5]. At the time of publication, only one anti-obesity drug, orlistat (tetrahydrolipstatin), was available, with two more approved recently [6]. Although antidiabetic agents that have become available in recent years, such as the glucagon-like peptide-1 (GLP-1) agonists and an amylin analog, are associated with weight loss [3, 4], there is still a need for drugs that can effectively achieve both glycemic control and weight control in T2DM patients.
α-glucosidase inhibitors (AGIs) prevent the digestion of disaccharides in the brush-border membranes of the small intestine and thereby effectively suppress the postprandial hyperglycemia observed in T2DM patients [7]. Three AGIs are currently available: acarbose, voglibose, and miglitol. Although AGIs are considered weight neutral [3, 4], multiple clinical studies indicate that AGIs not only lower blood glucose levels but are also associated with a lower body mass index in patients [7, 8]. Miglitol (a 1-deoxynojirimycin derivative) is the only AGI that is absorbed in the small intestine and enters the bloodstream [9]; anecdotally, it is more effective than other AGIs at decreasing the body weight of patients. Recent reports indicate that miglitol reduces visceral fat accumulation in patients with metabolic syndrome and body weight in T2DM patients, whereas voglibose fails to do so [10, 11]. Miglitol has been reported to increase postprandial GLP-1 levels in humans, and it is associated with increased satiation [12], offering a possible explanation for its anti-obesity effects. However, the precise mechanism of the weight-lowering effects of miglitol has not been addressed.

To determine the mechanism of the weight-lowering effects of miglitol, we investigated whether miglitol prevents diet-induced obesity in mice who were fed a high-fat high-sucrose (disaccharide composed of glucose and fructose) diet and a high-fat high-glucose/fructose diet. We found that miglitol prevents diet-induced obesity by stimulating energy expenditure in both diets, indicating that the effect is independent of preventing carbohydrate digestion in the gastrointestinal tract. Because the nonabsorbable AGI acarbose failed to stimulate energy expenditure in mice, we hypothesized that circulating miglitol stimulates brown adipose tissue (BAT). Therefore, we analyzed the effect of miglitol on the HB2 brown adipose cell line and found that miglitol enhances adrenergic signaling in brown adipocytes in vitro. Based on these data, miglitol is effective in treating hyperglycemia and obesity through two different mechanisms, providing the advantage of addressing both T2DM per se and obesity, a major risk factor for T2DM.

Materials and Methods

Animal studies

All animal care and experimental procedures were approved by the Institutional Animal Care and Experimentation Committee at Gunma University. All animal experiments described in the manuscript were conducted in accordance with accepted standards of humane animal care as outlined in the Ethical Guidelines. Mice were housed in individual cages in a temperature-controlled facility with a 12-h light/dark cycle. Mice were allowed free access to water and were given diet as indicated in each study. Mice were fed standard laboratory chow diet (CLEA Rodent diet CE-2; CLEA Japan, Tokyo, Japan) and a high-fat high-sucrose diet (F2HFHSD, Oriental Yeast, Saitama, Japan) with or without either miglitol (400 ppm or 800 ppm) or acarbose (800 ppm). A high-fat high-glucose/fructose diet (HFHGFD) was specially prepared by replacing sucrose with glucose and fructose (Oriental Yeast, Suita, Japan). Either a multifeeder (MF-1M; SHINFACTORY, Fukuoka, Japan) or a BioDAQ cage (Research Diets, New Brunswick, NJ, USA) was used for measuring food intake. C57Bl/6 male mice were purchased from CLEA Japan. Miglitol was provided by Sanwa Chemical Industry Company (Nagoya, Japan).

Feeding protocols

(i) Six-week miglitol feeding

Twelve-week-old C57Bl/6 mice were fed HFHSD with or without 800 ppm miglitol for 6 wk. Body weight and food intake were measured once a week.

(ii) Fourteen-week miglitol feeding

Four-week-old C57Bl/6 mice were fed HFHSD with or without miglitol (400 or 800 ppm) for 14 wk. Body weight and food intake were measured once a week.

(iii) HFHSD vs. HFHGFD study.

Twelve-week-old C57Bl/6 mice were fed HFHSD or HFHGFD with or without 800 ppm miglitol for 2 wk. Body weight and food intake were measured once a week.

After the study period in studies (i)-(iii), the mice were subjected to indirect calorimetry and locomotor activity analyses. Subsequently, they were killed and their plasma, epididymal WAT, interscapular BAT, and soleus were harvested.

(iv) Intraperitoneal miglitol injection study

Eleven-week-old C57Bl/6 mice were acclimated to 4-h restricted feeding (1800 h to 2200 h) with a standard chow diet for 1 wk. The diet was then switched from standard chow to HFHSD, and the mice were subjected to indirect calorimetry. After 4-day acclimation to indirect calorimetry and 4-h restricted feeding, the mice received an intraperitoneal injection of 0.1 mL of phosphate-buffered saline with or without 0.5 mg of
miglitol at 1800 h, 1900 h, 2000 h, and 2100 h. The dose of injected miglitol was calculated based on the average daily intake of HFHSD with 800 ppm miglitol (2.5 g/day × 800 ppm = 2 mg/d).

(v) Six-week acarbose feeding
We first compared the effect of acarbose and miglitol on preventing post-prandial hyperglycemia and determined that 800 ppm acarbose is equivalent to 800 ppm miglitol with regard to glycemic control (data not shown). We then fed 12-wk-old C57Bl/6 mice HFHSD with or without 800 ppm acarbose for 6 wk. Body weight and food intake were measured once a week. After the study period, the mice were subjected to indirect calorimetry and locomotor activity analyses. Stools were collected after the indirect calorimetry and dried for structural analysis. Subsequently, mice were killed, their abdomen inspected, and their intestines analyzed for distention.

Indirect calorimetry and locomotor activity measurement
Oxygen consumption and CO2 production were measured in individual mice at the indicated age with an Oxymax apparatus (Columbus Instruments, Columbus, OH, USA). O2 and CO2 measurements were performed every 18 min for each animal over a 3-5-d period. The respiratory quotient was calculated by dividing CO2 production (VCO2) by O2 consumption (VO2). Heat generation was calculated as:

heat = calorie value × VO2
(calorie value = 3.815 + 1.232 × VCO2/VO2).

Each calculation was adjusted to account for the individual mouse’s body weight. Locomotor activity was measured with the ACTIMO-100 (SHINFACTORY, Fukuoka, Japan).

Histological studies
Adipose tissues were fixed in 4% paraformaldehyde and embedded in paraffin. Paraffin sections were stained with hematoxylin and eosin or with anti-UCP1 antibody (ab10983; Abcam, Cambridge, MA, USA). Adipocyte size was measured as described previously [13].

Quantitative reverse transcription PCR
RNA was isolated from tissues with RNAsio plus (TaKaRa Bio, Otsu, Japan). Complementary DNA was made with the Improm II Reverse Transcription system (Promega, Tokyo, Japan). Real-time PCR was performed with the LightCycler system and LightCycler 480 SYBR Green I (Roche Diagnostics, Tokyo, Japan). The following primers were used: Actb (NM_007393), forward AGCCTTCTTTCTGGGTA, reverse GAGCAATGATCTTGATCTTC; Cebpa (NM_007678), forward AACAAACGCAACGTGGAGA, reverse GCCGTATTGTCACTGGTC; Fabp4 (NM_024406), forward AGAAGTGAGGATGGCTTTGCCA, reverse CAGGGCCCCCCCATCTAGGG; Pparg (NM_011146), forward AGGGCCGAGAGGAGAGCTGTG, reverse TGGCCACCTTTGTCTGCTC; Ppargc1a (NM_008904), forward AATTCTCCCTTGATGTGAGA, reverse TTACCTCCGCAAGCTTTCTG; Prdm16 (NM_027504), forward AAGCAGCGGAGGCCGC, reverse CCAGGTCTTGGACTGTTT; and Ucp1 (NM_009463), forward TACAGAGTTATAGCACCACA, reverse CATGATGACGTCCA.

Western blot analysis
Cell lysates were prepared as described previously [14]. The following antibodies used for western blotting were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) [α-tubulin (sc-5286)] or from Cell Signaling (Danvers, MA, USA) [phospho-CREB (Ser133) (#9198), CREB (#9197), phospho-ATF2 (Thr69/71) (#9225), ATF2 (#9226), phospho-HSL (Ser563) (#4139), and HSL (#4107)]. Signals were visualized with an LAS4000 imager (FUJIFILM, Tokyo, Japan).

ELISA measurement of plasma GLP-1
Plasma active GLP-1 levels were measured with the GLP-1 (Active) ELISA Kit (#AKMGP-011; Shibayagi, Shibukawa, Japan).

Differentiation of HB2 cells
HB2 preadipocytes were differentiated as previously described [15]. Briefly, HB2 cells were seeded on a collagen-coated plate and cultured with D-MEM high glucose supplemented with 10% FBS until confluence. Then, 0.5 mM 3-isobutyl-1-methylxanthine (IBMX) and 1 µM dexamethasone were added to induce differentiation. Next, cells received 10 µg/mL insulin and 50 nM triiodothyronine (T3) on days 2, 4, and 6. For catecholamine stimulation experiments, differentiated HB2 cells were cultured in serum-free D-MEM for 24 h (days 8 to 9) and treated with miglitol and isoprotrenol. For gene expression analyses, HB2 cells were differentiated in the presence or absence of miglitol from day 0 to 8 and harvested on day 8.
Transfection of plasmid DNA
Differentiated HB2 adipocytes grown on a 100-mm culture dish were dispersed with PBS containing 0.5 mg/mL Collagenase Type II (Worthington Biochemical Corporation, Lakewood, NJ, USA) and 0.05% trypsin. After washing five times with PBS, cells were resuspended in Electroporation Buffer (Bio-Rad, Hercules, CA, USA). An aliquot of cells (0.55 mL) was mixed with 0.2 mL of the Epacl-camps plasmid [16] (30 µg) in a 0.4-cm gap cuvette before a single pulse of electroporation with the Gene Pulser Xcell (Bio-Rad) set at voltage 200 V and time constant 28 ms. Electroporated cells were resuspended in growth medium and seeded on a 35-mm glass-bottom culture dish (MatTek, Ashland, MA, USA).

Measurement of cytosolic cAMP
Before measurements, the growth medium was removed and washed twice with Hanks' balanced salt solution (HBSS) containing 1.3 mM CaCl₂, 5.4 mM KCl, 0.44 mM KH₂PO₄, 0.5 mM MgCl₂, 0.38 mM MgSO₄, 138 mM NaCl, 0.34 mM Na₂HPO₄, 5.5 mM D-glucose, and HEPES/NaOH (pH 7.4). After washing, cells were resuspended in HBSS containing 0, 10, 50, or 100 µM miglitol and incubated for 20 min. Cells were visualized with a 40 UApo/340 objective lens (Olympus, Tokyo, Japan). To detect fluorescence images, we used AQUACOSMOS/ASHURA, a 3CCD-based fluorescence energy transfer imaging system (Hamamatsu Photonics, Hamamatsu, Japan). Images (100 ms exposure) were captured with a C7780-22 ORCA3CCD camera (Hamamatsu Photonics) at 10-s intervals.

Differentiation of 3T3-L1 cells
The 3T3-L1 preadipocytes were differentiated as previously described with AdipoInducer Reagents (TaKaRa Bio) [17]. Briefly, 3T3-L1 cells were seeded on a collagen-coated plate and cultured with D-MEM high glucose supplemented with 10% FBS until confluence. Then, insulin (5 µg/mL), IBMX (0.5 mM), and dexamethasone (2.5 µM) were added to induce differentiation. Next, cells received 5 µg/mL insulin on days 2, 4, and 6. For catecholamine stimulation experiments, differentiated 3T3-L1 cells were cultured in serum-free D-MEM for 24 h (day 8 to 9) and treated with miglitol and isoproterenol. For gene expression analyses, oil red O staining, and glycerol assays, 3T3-L1 preadipocytes were differentiated in the presence or absence of miglitol from days 0 to 8 and harvested on day 8. Oil red O staining was performed as previously described [18]. For the glycerol assay, 3T3-L1 preadipocytes were differentiated in 96 wells and 10 µL of the supernatants from the differentiated 3T3-L1 adipocytes were analyzed on day 8 with the Glycerol Colorimetric Assay Kit (Cayman Chemicals, Ann Arbor, MI, USA).

Statistical analysis
Data are expressed as the mean value ± SEM. Statistical significance was assessed using Student’s t-test unless written otherwise. P-values < 0.05 were considered statistically significant.

Results
Miglitol prevents diet-induced obesity by stimulating energy expenditure and inducing mitochondrial gene expression in brown adipose tissue
In order to first confirm the anti-obesity effect of miglitol in mice, 12-wk-old C57Bl/6 male mice were fed HFHSD alone or HFHSD mixed with 800 ppm miglitol (HFHSD+M) for 6 wk. Weight gain caused by HFHSD was significantly prevented in the miglitol-fed group (Fig. 1A). The attenuated weight gain was accompanied by a decrease in the weight of epididymal white adipose tissue (eWAT) (Fig. 1B). Histologically, the size of the adipocytes in eWAT was significantly smaller in the miglitol-fed mice than in the control mice (Fig. 1C, D).

To determine how miglitol prevents diet-induced obesity, the food intake, oxygen consumption, and locomotor activities of the mice were analyzed. Although there was no difference in food intake between the control and miglitol-fed groups (Fig. 2A), the miglitol-fed mice had significantly higher oxygen consumption after 6 wk of miglitol feeding (Fig. 2B, C). Meanwhile, miglitol did not affect the locomotor activity of the mice (Fig. 2D). Therefore, increased energy expenditure was the primary reason for the prevention by miglitol of diet-induced obesity in mice.

We next fed 4-wk-old male C57BL/6 mice with HFHSD or HFHSD+M for 14 wk with two different doses of miglitol (400 ppm and 800 ppm). Miglitol dose-dependently suppressed weight gain (Fig. 3A). The long-term miglitol treatment significantly increased the expression of Ppargc1a and Ucp1 in brown adipose tissue (BAT) but not in soleus muscle of miglitol-fed mice (Fig. 3B, C). Miglitol increased
Miglitol stimulates energy expenditure

**Fig. 1** Miglitol (M) prevents diet-induced obesity
(A) Body weight over the course of the study. (B) Epididymal white adipose tissue (eWAT) weight at 18 wk of age. (C) Representative hematoxylin and eosin staining of eWAT of high-fat high-sucrose diet (HFHSD)-fed mice (upper) and HFHSD+miglitol (M)-fed mice (lower). Magnification: ×40 (D) Size distribution of eWAT adipocytes. Black indicates data from HFHSD-fed mice (n = 8), and gray indicates data from HFHSD+M-fed mice (n = 8). Statistical analyses were performed with two-tailed unpaired Student’s t-test (*, p < 0.05; **, p < 0.01; and ***, p < 0.001).

**Fig. 2** Miglitol (M) prevents diet-induced obesity by stimulating energy expenditure
(A) Food intake over the course of the study. (B–C) Oxygen consumption trend over 24-h period (B), and 24-h cumulative oxygen consumption (C) of mice at 18 wk of age. (D) Locomotor activity of mice during indirect calorimetry at 18 wk of age. Black indicates data from high-fat high-sucrose diet (HFHSD)-fed mice (n = 8), and gray indicates data from HFHSD+M-fed mice (n = 8). Statistical analyses were performed with two-tailed unpaired Student’s t-test (*, p < 0.05).
plasma active GLP-1 levels in a dose-dependent manner, as expected (Fig. 3D). In summary, miglitol treatment induced mitochondrial gene expression in BAT, increased energy expenditure, and prevented diet-induced obesity in mice.

**Miglitol stimulates energy expenditure independent of preventing the digestion of carbohydrates in the gastrointestinal tract**

As an AGI, miglitol prevents post-prandial hyperglycemia by preventing the digestion of carbohydrates in the gastrointestinal tract. To test if the anti-obesity effect of miglitol was dependent on this mechanism, we prepared HFHGFD, which contains glucose and fructose equivalent to the sucrose in HFHSD. Because disaccharide sucrose is already broken down into glucose and fructose in HFHGFD, miglitol does not prevent the digestion and absorption of carbohydrate in the meal. We fed 12-wk-old C57BL/6 male mice with HFHSD with/without miglitol or HFHGFD with/without miglitol for 2 wk. Unexpectedly, miglitol was more effective at preventing diet-induced obesity with HFHGFD feeding than with HFHSD feeding (Fig. 4A, B). Miglitol increased oxygen consumption in the HFHGFD-fed mice more effectively than in the HFHSD-fed mice (Fig. 4C). There was no significant difference in locomotor activity between these mice (data not shown). Mice consumed more HFHGFD
Miglitol stimulates energy expenditure

Intraperitoneal injection of miglitol was sufficient to stimulate energy expenditure in mice. Compared to the vehicle injection, intraperitoneal injection of miglitol (0.5 mg × four injections, one injection each hour) over the course of 4 h during restricted feeding significantly stimulated the oxygen consumption of the mice (Fig. 4E). Locomotor activity was not different between the two groups (data not shown). These data indicate that miglitol that enters the bloodstream can stimulate energy expenditure in vivo.

Fig. 4 Miglitol (M) stimulates energy expenditure independent of preventing the digestion of carbohydrates

(A–D) Miglitol prevents diet-induced obesity by stimulating energy expenditure in mice fed a high-fat high-glucose/fructose diet. Percent change in body weight over the course of the study (A) and eWAT weight after 2 wk (B). Twenty-four-hour oxygen consumption after 2 wk (C), and food intake over the course of the study (D). Black, high-fat high-sucrose diet (HFHSD) only; gray, HFHSD + miglitol; black stripe, high-fat high-glucose/fructose diet (HFHGFD) only; and gray stripe, HFHGFD + miglitol (n = 10 each). Statistical analyses were performed to compare the effect of miglitol between HFHSD only vs. HFHSD+M and between HFHGFD only vs. HFHGFD+M with two-tailed unpaired Student’s t-test (*, p < 0.05). For (D), two-way ANOVA was performed to assess the effect of diet and miglitol on food intake (data in the Results). (E) Trend and 4-h cumulative oxygen consumption of mice that received intraperitoneal injections of miglitol. Mice under 4-h restricted feeding schedules (between 1800 h and 2200 h) received four intraperitoneal injections of 100 µL of either PBS or miglitol (0.5 mg/shot) every hour starting at 1800 h. Black, PBS injection (n = 8) and gray, miglitol injection (n = 8). Statistical analyses were performed with two-tailed unpaired Student’s t-test (*, p < 0.05).

than HFHSD regardless of the presence of miglitol in the diet (two-way ANOVA, p < 0.05 at 12 wk and p < 1 × 10⁻⁵ at 13 and 14 wk; Fig. 4D), resulting in increased miglitol intake per day in the HFHGFD+M-fed group, which is probably the reason why a stronger anti-obesity effect was observed with HFHGFD feeding. These data indicate that miglitol stimulates energy expenditure independent of preventing the digestion of carbohydrates in the GI tract.

Miglitol is the only AGI that is absorbed in the small intestine and enters the bloodstream [9]. To obtain further supporting evidence that the increased energy expenditure observed in miglitol-fed mice is independent of its effect in the GI tract, we tested if intraperitoneal injection of miglitol was sufficient to stimulate energy expenditure in mice. Compared to the vehicle injection, intraperitoneal injection of miglitol (0.5 mg × four injections, one injection each hour) over the course of 4 h during restricted feeding significantly stimulated the oxygen consumption of the mice (Fig. 4E). Locomotor activity was not different between the two groups (data not shown). These data indicate that miglitol that enters the bloodstream can stimulate energy expenditure in vivo.

**Acarbose does not stimulate energy expenditure, but causes indigestion and prevents diet-induced obesity**

To test if miglitol’s ability to enter the bloodstream
Physiological doses of miglitol promote adrenergic signaling in brown adipocytes in vitro

Because long-term miglitol treatment stimulated mitochondrial gene expression in BAT but not in skeletal muscle (Fig. 3B, C), we hypothesized that miglitol in the circulation directly stimulates BAT and enhances energy expenditure. PGC-1α regulates the expression of UCP1, and beta-adrenergic receptor (βAR) signaling regulates the expression of PGC-1α by activating the cAMP-Protein kinase A (PKA) pathway and inducing phosphorylation of CREB and ATF2, which bind to and activate the PGC-1α promoter [19]. To test the direct effect of miglitol on βAR-cAMP-PKA signaling, immortalized brown preadipocyte HB2 cells were differentiated into mature brown adipocytes [15]. Pretreatment of HB2 cells with 50-100 µM miglitol for 15-30 min enhanced the response to 1-10 µM isoprotrenol stimulation, which resulted in increased intracellular cAMP levels (Fig. 6A, B) and enhanced phosphorylation of CREB and the downstream target ATF2 (Fig. 6C). Miglitol also had a mild effect on the baseline phosphorylation of CREB in the absence of isoproterenol stimulation (Fig. 6C). Although miglitol did not affect the expression of Prdm16, the master regulator of brown adipocyte differentiation [20], 5 mM miglitol significantly increased PGC-1α and UCP1 expression (Fig. 6D). These data indicate that physiologically relevant dosages of miglitol enhance βAR signaling in brown adipocytes in vitro, and they are consistent with increased PGC-1α and UCP1 expression in the BAT of miglitol-fed mice in vivo.

A pharmacological dose of miglitol is required to affect adrenergic signaling in white adipocytes in vitro

We also tested the effect of miglitol on 3T3-L1 white adipocyte cells. Enhanced phosphorylation of hormone-sensitive lipase (HSL), another target of βAR-cAMP-PKA signaling [21-23], was not observed in differentiated 3T3-L1 white adipocytes after treatment with 50-100 µM miglitol (Fig. 7A). Meanwhile, the presence of millimolar doses of miglitol prevented lipid accumulation during 3T3-L1 differentiation (Fig. 7B). Because a single oral dose of 50 mg or 100 mg of miglitol results in maximum plasma concentrations of 6.27 µM or 8.4 µM, respectively, in humans [24, 25], the effects seen with micromolar doses of miglitol treatments are physiologically relevant, whereas those seen with millimolar doses of miglitol are likely to be pharmacological effects. A millimolar dose of miglitol did not affect the expression levels of the white adipocyte differentiation markers Pparg, Cebpa, and Fabp4 (Fig. 7C), but it did stimulate lipolysis (Fig. 7D). Therefore, the decreased lipid content in the differentiated 3T3-L1 cells was not due to the suppression of white adipocyte differentiation, but due to increased lipolysis. The effect on lipolysis was not observed with 50-100 µM doses of miglitol (data not shown).

In summary, physiologically relevant doses of miglitol promote βAR-cAMP-PKA signaling in brown adipocytes, but not in white adipocytes, in vitro. It is likely that the decreased adiposity observed in miglitol-fed mice was due to increased energy expenditure caused by miglitol acting on BAT, but not due to increased lipolysis caused by miglitol acting on WAT, in vivo.

Discussion

In the current study, our aim was to uncover the mechanism behind the weight-lowering effects of miglitol. We found that miglitol prevents diet-induced obesity in mice by stimulating energy expenditure independent of preventing the digestion of carbohydrates. The weight-decreasing effect of miglitol via the stimulation of energy expenditure was also observed in mice fed a high-fat high-glucose/fructose diet, which contains monosaccharides instead of a disaccharide. The facts that intraperitoneal injection of miglitol was sufficient to increase the energy expenditure and that
Miglitol stimulates energy expenditure

Acarbose does not stimulate energy expenditure, but causes indigestion and prevents diet-induced obesity

(A) Body weight over the course of the study. (B) Epididymal white adipose tissue (eWAT) weight at 18 wk of age. (C) Food intake over the course of the study. (D–E) Oxygen consumption trend over 24-h period (D), and 24-h cumulative oxygen consumption (E) of mice at 18 wk of age. (F) Locomotor activity of mice during indirect calorimetry at 18 wk of age. (G) Representative images of abdomen (upper panels), intestine (middle panels), and dried stools (lower panels) of mice fed either HFHSD, HFHSD with miglitol, or HFHSD with acarbose. Black indicates data from HFHSD-fed mice (n = 7), and gray indicates data from HFHSD+A-fed mice (n = 9). Statistical analyses were performed with two-tailed unpaired Student’s t-test (*, p < 0.05 and **, p < 0.01).
Fig. 6  Physiological doses of miglitol promote adrenergic signaling in brown adipocytes in vitro
(A–B) Intracellular cAMP levels of differentiated HB2 cells after 1 µM isoproterenol stimulation in the presence of miglitol measured by the Epac1-camps sensor. The CFP/YFP fluorescence ratio represents intracellular cAMP levels (n = 4 per group). (B) Area under the curve of CFP/YFP values from 10 to 320 s after isoproterenol stimulation. (C) Western blots of differentiated HB2 cells with or without miglitol pretreatment before or 30 min after the 10 µM isoproterenol stimulation. Note that increased phosphorylation of ATF-2 caused an upward band shift, which is enhanced by miglitol pretreatment. (D) Effect of miglitol on expression levels of Prdm16, Ppargc1a, and Ucp1 during HB2 differentiation measured by quantitative RT-PCR (n = 3 per group). Statistical analyses were performed with one-way ANOVA followed by two-tailed unpaired Student’s t-test (*, p < 0.05; **, p < 0.01; and ***, p < 0.001).

Fig. 7  Pharmacological doses of miglitol are required to affect adrenergic signaling in white adipocytes in vitro
(A) Western blots of differentiated 3T3-L1 cells with or without miglitol pretreatment before or 15, 30, or 60 min after 10 µM isoproterenol stimulation. (B) Images of 3T3-L1 cells after oil red O staining (40× magnification). (C) Effect of 5 mM miglitol on expression levels of Pparg, Cebpa, and Fabp4 during 3T3-L1 differentiation measured by quantitative RT-PCR (n = 6 per group). (D) Effect of miglitol on glycerol release from differentiated 3T3-L1 adipocytes (n = 4 per group). Statistical analyses were performed with one-way ANOVA followed by two-tailed unpaired Student’s t-test (*, p < 0.05; **, p < 0.01; and ***, p < 0.001).
acarbose (an AGI that does not enter the bloodstream) did not increase energy expenditure in mice support the idea that miglitol in the circulation has a direct role in stimulating energy expenditure. Miglitol administered orally is almost completely absorbed; it enters the circulation with very low permeation across the blood/brain barrier and is excreted via the kidney without any apparent metabolism within the body [26]. Intravenous injection of miglitol reduces the extent of myocardial infarcts in rabbits [27], indicating that miglitol in the bloodstream can have biological effects in peripheral tissues.

The mechanism that we uncovered is different from the assumption commonly held by many clinicians, which is that miglitol treatment leads to the suppression of appetite by increasing plasma GLP-1 levels. GLP-1 is well known to suppress food intake [28], but there is no clear evidence as to whether it stimulates energy expenditure. Blocking brain GLP-1 signaling by exendin-9 for 1 month dramatically increases food intake without causing weight gain, suggesting that brain GLP-1 signaling negatively regulates energy expenditure [29]. Therefore, increased energy expenditure is likely to be independent of the GLP-1 effect.

Intraperitoneal injection of miglitol stimulated energy expenditure in mice that had been accustomed to a 4-h restricted feeding protocol. The effect was not clear in ad lib-fed mice (data not shown). Therefore, miglitol had to be in the bloodstream while mice were feeding in order for us to clearly observe the effect on increasing energy expenditure. Because meal consumption stimulates the sympathetic activity of BAT and leads to diet-induced thermogenesis [30], feeding and circulating miglitol may act synergistically to enhance energy expenditure.

There were differences in the degree of response to miglitol between brown adipocytes and white adipocytes; a physiologically relevant micromolar concentration of miglitol was enough to enhance the response to catecholamine in HB2 brown adipocytes, whereas a millimolar concentration of miglitol was necessary to see any effect in 3T3-L1 white adipocytes. A significant effect on mitochondrial gene expression was only seen in the BAT of mice fed miglitol for a long-term period, indicating that high sympathetic activity within BAT and chronic miglitol treatment synergistically promotes βAR-cAMP-PKA signaling in BAT, leading to increased expression of PGC-1α and UCP1. Meanwhile, the enhanced “browning” of inguinal WAT was not observed in miglitol-fed mice reared at ambient temperature (data not shown). WAT contains 25 times less noradrenaline compared to BAT, indicating that there is less sympathetic innervation to WAT [31]. A further increase in sympathetic activity in subcutaneous WAT (e.g., by cold stimulation) may be required for miglitol to synergistically promote the “browning” of subcutaneous WAT in miglitol-fed mice.

Miglitol exerted an effect on the cAMP-signaling pathway within 15-30 min after treatment, indicating that the effect is unlikely to depend on transcription. Although neither a cell-surface receptor nor a transporter for miglitol has been identified, miglitol may bind to an unidentified Gs G protein-coupled receptor and mildly increase the intracellular cAMP level, or it may inhibit the activity of phosphodiesterases either directly or indirectly. Further investigation is necessary to identify the molecular target(s) that carry out the anti-obesity effect of miglitol.

The presence of metabolically active functional BAT was “rediscovered” in adult humans [32-35]. Both classical BAT and so-called beige/brite BAT are present in adult humans [36, 37], although others claim that all human BAT is beige/brite [38]. Sympathetic activity is required for the normal development and maintenance of BAT [39, 40]. The presence of BAT decreases with age in humans, and the existence of cold-activated BAT negatively correlates with body mass index and adiposity in humans [32, 41]. Aged C57BL/6J mice do respond to cold by increasing sympathetic nervous activity in interscapular BAT; thus, it is likely to be the sensitivity of BAT to adrenergic stimuli that decreases with age [42]. Therefore, miglitol may promote the maintenance of functional BAT by promoting beta-adrenergic signaling in BAT even in aging humans, who are expected to have less or no BAT.

The current study revealed a novel mechanism by which miglitol prevents diet-induced obesity; i.e., by stimulating BAT and enhancing energy expenditure independent of preventing carbohydrate digestion. We also showed that miglitol promotes adrenergic signaling in brown adipocytes. Considering that several anti-obesity drugs with central actions have been withdrawn from the market due to serious side effects, the weight-lowering mechanism of miglitol via peripheral tissue actions may be useful in the development of a new class of anti-obesity drug. Therefore, future investigations to elucidate the molecular target of miglitol in brown adipose tissue would be of interest and importance.
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Miglitol stimulates energy expenditure


