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Treatment with the α-glucosidase inhibitor miglitol from the preonset stage in Otsuka Long-Evans Tokushima Fatty rats improves glycemic control and reduces the expression of inflammatory cytokine genes in peripheral leukocytes

Kazuki Mochizuki[a], *Nanae Fukaya*[a], *Yutaro Tanaka*[a], *Masahiro Fuchigami*[b], *Toshinao Goda*[a,*]

[a] *Laboratory of Nutritional Physiology, Graduate School of Nutritional and Environmental Sciences and the Global COE program, The University of Shizuoka, Shizuoka 422-8526, Japan*
[b] *Pharmaceutical Research Laboratories, Sanwa Kagaku Kenkyusho, Ltd, Mie 511-0406, Japan*

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ABSTRACT

Otsuka Long-Evans Tokushima Fatty (OLETF) rats, an animal model of type 2 diabetes mellitus, exhibit chronic and slowly progressive hyperglycemia with obesity. In this study, we examined whether dietary supplementation with the α-glucosidase inhibitor miglitol from the preonset stage improves glycemic control and reduces the gene expression of inflammatory cytokines in peripheral leukocytes. The OLETF rats were fed a control diet or a diet containing 800 ppm miglitol (miglitol diet) for 40 weeks from 5 weeks of age (preonset stage). We determined nonfasting blood glucose, blood 1,5-anhydroglucitol, and messenger RNA levels of inflammatory cytokines in peripheral leukocytes in these rats. Nonfasting blood glucose concentrations gradually increased in OLETF rats fed the control diet, with significant increases at weeks 28 and 40 compared with week 0. In contrast, nonfasting blood glucose levels did not increase in miglitol-treated rats during the experimental period. Miglitol-treated rats had lower nonfasting blood glucose levels and higher 1,5-anhydroglucitol levels, a marker for glucose fluctuations, at week 40 than control rats. The gene expression of inflammatory cytokines including interleukin-6, tumor necrosis factor-α, and interferon-γ in peripheral leukocytes gradually increased during the development of diabetes in control rats, but not in miglitol-treated rats. Our results suggest that dietary supplementation with miglitol from the preonset stage in OLETF rats improves glycemic control and reduces gene expression of cytokines related to inflammation in peripheral leukocytes.

K. Mochizuki wrote the manuscript, did statistical analysis, and contributed to the experiments; N. Fukaya, Y. Tanaka, and M. Fuchigami contributed to the experiments; and T Goda organized the study.

* *Corresponding author*. Department of Nutrition, School of Food and Nutritional Sciences, The University of Shizuoka, Shizuoka-shi, Shizuoka 422-8526, Japan. Tel.: +81 54 264 5533; fax: +81 54 264 5565.
E-mail address: gouda@u-shizuoka-ken.ac.jp (T. Goda).

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1. Introduction

Hyperglycemia in diabetes induces many complications such as nephropathy, decreased pancreatic insulin secretion, insulin resistance, retinopathy, and cardiovascular diseases [1]. These hyperglycemia-related complications are principally caused by inflammatory activities, which include oxidative stress, viral/bacterial infection, and activation of leukocytes such as monocytes/macrophages [1]. Recent studies have shown that hyperglycemia directly induces inflammation by enhancing the expression of inflammatory cytokines such as interleukin (IL)-1β, IL-6, IL-12, IL-18, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ. These cytokines are mainly expressed by leukocytes, including macrophages, monocytes, and neutrophils, and by many peripheral tissues [2-4]. These cytokines activate macrophages/monocytes and induce the infiltration of macrophages into many tissues [2]. Several studies have also shown that the cytokines that are enhanced by hyperglycemia directly induce inflammation without the infiltration of macrophages in many tissues. For example, several cytokines induce apoptosis in islet β-cells by activating the caspase cascade, resulting in the onset of diabetes because of decreased insulin secretion from the islet β-cells [5]. In addition, these cytokines induced by hyperglycemia enhance macrophage infiltration to the vascular endothelium, thus increasing the risk of atherosclerosis [6,7]. One of the major causes of insulin resistance is thought to be the production of cytokines because exposing adipocytes to cytokines induces insulin resistance in the cells [8]. Indeed, the circulating protein concentrations of IL-1β, IL-6, IL-12, IL-18, TNF-α, and IFN-γ are higher in patients with type 2 diabetes mellitus than in healthy subjects [9-12]. Thus, it is thought that controlling glycemic status is important to prevent the progression of diabetes and related complications caused by cytokine production and activated leukocytes. Recent epidemiological studies have shown that postprandial hyperglycemia, rather than hemoglobin A_{1c}, is associated with increased mortality and the risk of developing cardiovascular diseases, including arteriosclerosis, in people with diabetes [13]. Furthermore, the epidemiological Study to Prevent Non–Insulin-Dependent Diabetes Mellitus and the Meta-analysis of Risk Improvement under Acarbose-7 trials revealed that inhibition of postprandial hyperglycemia with acarbose, an α-glucosidase inhibitor, reduced the incidence of diabetes and related complications such as hypertension and cardiovascular disease in patients with impaired glucose tolerance or overt type 2 diabetes mellitus [1,14-16]. Considering that abnormal inflammatory cytokine production is the major cause of diabetes and its complications, inhibiting postprandial hyperglycemia using an α-glucosidase inhibitor may prevent the development and progression of diabetes and its complications by suppressing the expression of inflammatory cytokines. Our recent studies have demonstrated that dietary supplementation with an α-glucosidase inhibitor, miglitol, for 4 weeks reduced the expression of inflammatory cytokines such as IL-1β and TNF-α in peripheral leukocytes from streptozotocin-induced diabetic rats [17]. However, it is still unclear whether inhibition of postprandial hyperglycemia from the preonset stage reduces the expression of inflammatory cytokines in peripheral leukocytes because this animal model exhibits severe acute diabetes, as streptozotocin causes extensive pancreatic islet β-cell destruction.

In the present study, we examined whether inhibition of postprandial hyperglycemia by miglitol improves glycemic control and reduces the expression of inflammatory cytokine genes in peripheral leukocytes. To achieve this, we used Otsuka Long-Evans Tokushima Fatty (OLETF) rats starting at the preonset stage, an animal model that exhibits chronic and slowly progressive hyperglycemia and hyperlipidemia with obesity due to overeating, similar to that in humans [18].

2. Materials and methods

2.1. Animals

Five-week-old male OLETF rats, which were obtained at 4 weeks old from the Otsuka GEN Research Institute (Tokushima, Japan), were assigned to 2 groups. The first group was given a control diet, which was based on the rodent diet for experiments determined by the American Institute of Nutrition (AIN) [19] and contains 20% (wt/wt) casein, 40.5% corn starch, 16% sucrose, 8% corn oil, 5.4% lard, 3.5% AIN93 vitamin mix, 1% AIN93G mineral mix, 5% cellulose, 0.3% L-cystine, and 0.2% choline bitartrate. The second group of rats was given the same diet supplemented with 800 ppm (0.8 g/1000 g diet) miglitol (miglitol diet), which was provided by Sanwa Kagaku Kenkyusho (Mie, Japan) (Table 1). The rats in both groups were fed the respective diet for 40 weeks. Rats were housed individually in cages and given free access to food and tap water. The rats were maintained at a stable temperature (22°C ± 2°C) and humidity (55% ± 5%) and a 12-hour light/dark cycle (light: 7:00 AM to 7:00 PM). At weeks 0, 16, 28, and 40, nonfasting plasma samples were collected from the tail vein using a capillary tube containing heparin-lithium between 9:30 and 10:30 AM. The experimental procedures used in the present study conformed to the guidelines of the Animal Usage Committee of the University of Shizuoka.

2.2. Parameters

Glycemic status was determined in terms of nonfasting blood glucose levels and 1,5-anhydroglucitol (1,5-AG) levels, a known marker of glucose fluctuations, because miglitol suppresses

Table 1 – Diet composition

	Control	Miglitol
α-Corn starch	405.3	405.3
Sucrose	160.2	160.2
Corn oil	80.4	80.4
Lard	53.6	53.6
Casein	200	200
AIN93G mineral mix	35	35
AIN93 vitamin mix	10	10
Choline bitartrate	2.5	2.5
L-Cystine	3	3
Cellulose	50	49.2
Miglitol	–	0.8
Total	1000	1000

postprandial glucose elevations. Thus, we collected nonfasting plasma from the OLETF rats. Plasma glucose and triacylglycerol levels were measured using commercial kits (Wako Pure Chemical Industries, Osaka, Japan). 1,5-Anhydroglucitol was assayed using a commercial kit (Determiner-L 1,5-AG; Kyowa Medex, Tokyo, Japan) on a Hitachi 7170S automatic analyzer (Hitachi High-Technologies, Tokyo, Japan).

2.3. *Real-time reverse transcriptase polymerase chain reaction*

Blood samples for RNA extraction were obtained from the tail vein at weeks 16, 28, and 40. Each sample was immediately mixed in a PAXgene RNA tube with a fixation solution, which immediately fixes the leukocytes without changing messenger RNA (mRNA) levels in these cells (Qiagen/BD, Tokyo, Japan). The blood samples were incubated in fixation solution at room temperature for 1 day and then stored at –70°C in accordance with the manufacturer's instructions. Total RNA was then extracted from blood samples using the PAXgene kit purchased from PreAnalytix (Qiagen/BD). Total RNA samples (100 ng) were converted to complementary DNA by reverse transcription using SuperScript II RT (Invitrogen, Tokyo, Japan) in accordance with the manufacturer's instructions. To quantitatively estimate the mRNA levels of the selected genes, polymerase chain reaction (PCR) amplification was performed on a Light-Cycler instrument (Roche Molecular Biochemicals, Tokyo, Japan), as previously described [20], using the PCR primer sequences listed in Table 2. We conducted real-time reverse transcriptase (RT)–PCR of several candidate genes, including YY1 transcriptional factor (YY1), transcription factor IIB, 18S rRNA, hypoxanthine phosphoribosyltransferase, and α-tubulin, to assess their suitability as an internal control. We chose YY1 as the internal control because its expression was the most invariable between the 2 experimental groups. The cycle threshold (CT) values of each gene and YY1 mRNA detected by real-time RT-PCR were converted into signal intensities by the Δ – Δ method [21], which calculates the difference of 1 CT value as a 2-fold difference between each signal for each gene and the signal for a gene for normalization (YY1). The formula used was $[2^{(CT\ YY1\text{-}CT\ each\ gene)}]$.

Table 2 – The sequences of oligonucleotide primers used in this study

	Sequence
IL-1β mRNA	5′-AAAATGCCTCGTGCTGTCTGA-3′
	5′-CAGGGATTTTGTCGTTGCTTG-3′
IL-6 mRNA	5′-CAGCGATGATGCACTGTCAGAA-3′
	5′-TCCAGGTAGAAACGGAACTCCA-3′
IL-12a mRNA	5′-TGAGGACGGCCAGAGAAAAAT-3′
	5′-AGGCACAGGGTCATCATCAAA-3′
IL-18 mRNA	5′-CAGACCACTTTGGCAGACTTCA-3′
	5′-ACACAGGCGGGTTTCTTTTGT-3′
TNF-α mRNA	5′-AGCATGATCCGAGATGTGGAA-3′
	5′-AATGAGAAGAGGCTGAGGCACA-3′
IFN-γ mRNA	5′-GCCAAGGCACACTCATTGAAA-3′
	5′-TTCGTGTTACCGTCCTTTTGC-3′
YY1 mRNA	5′-GAAGAGCAGATCATTGGGGAGA-3′
	5′-CAAATTCTGCCAGTTGCTTGG-3′

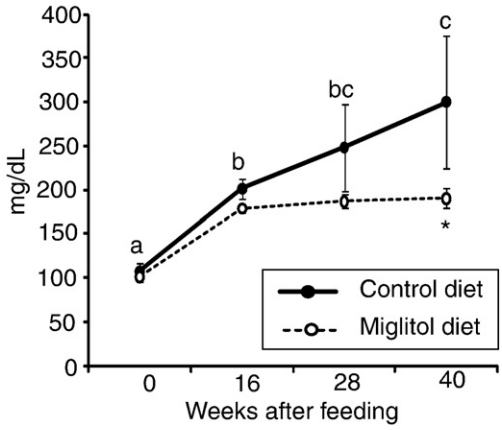

Fig. 1 – Effects of dietary supplementation with miglitol on nonfasting blood glucose concentrations in OLETF rats. Nonfasting blood glucose concentrations were determined at weeks 0, 16, 28, and 40. Values are means ± SEM (n = 7). *P < .05 vs the control group. Different letters (a-c) denote significant differences (P < .05) over time in each group.

2.4. *Statistics*

Results are expressed as means ± SEM. The significance of differences among groups was determined by Student *t* test (Table 2) or Tukey test based on 2-way repeated-measures analysis of variance (Figs. 1 and 2). $P < .05$ was considered to indicate statistical significance.

3. Results

3.1. *Body weight, food intake, and blood concentrations of glucose and 1,5-AG*

Body weight and food intake did not differ between the control and miglitol-treated OLETF rats throughout the experimental period. The blood glucose concentration in rats fed the control diet gradually increased during the experimental period and was significantly higher at weeks 16, 28, and 40 as compared with week 0. In contrast, the blood glucose concentration in rats fed the miglitol diet did not differ significantly during the experimental period and was lower at week 40 than in the control rats (Fig. 1). The 1,5-AG concentration was significantly higher in miglitol-treated rats at week 40 than in the age-matched control rats. The plasma triacylglycerol concentration did not differ between the 2 groups at week 40 (Table 3). Digestive symptoms, such as diarrhea, were not observed throughout the experimental period.

3.2. *Effects of miglitol on the expression of inflammatory cytokine genes in peripheral leukocytes*

To examine whether dietary supplementation with miglitol reduces the expression of inflammatory cytokine genes in the peripheral leukocytes of OLETF rats, we performed real-time RT-PCR using peripheral leukocyte RNA obtained from

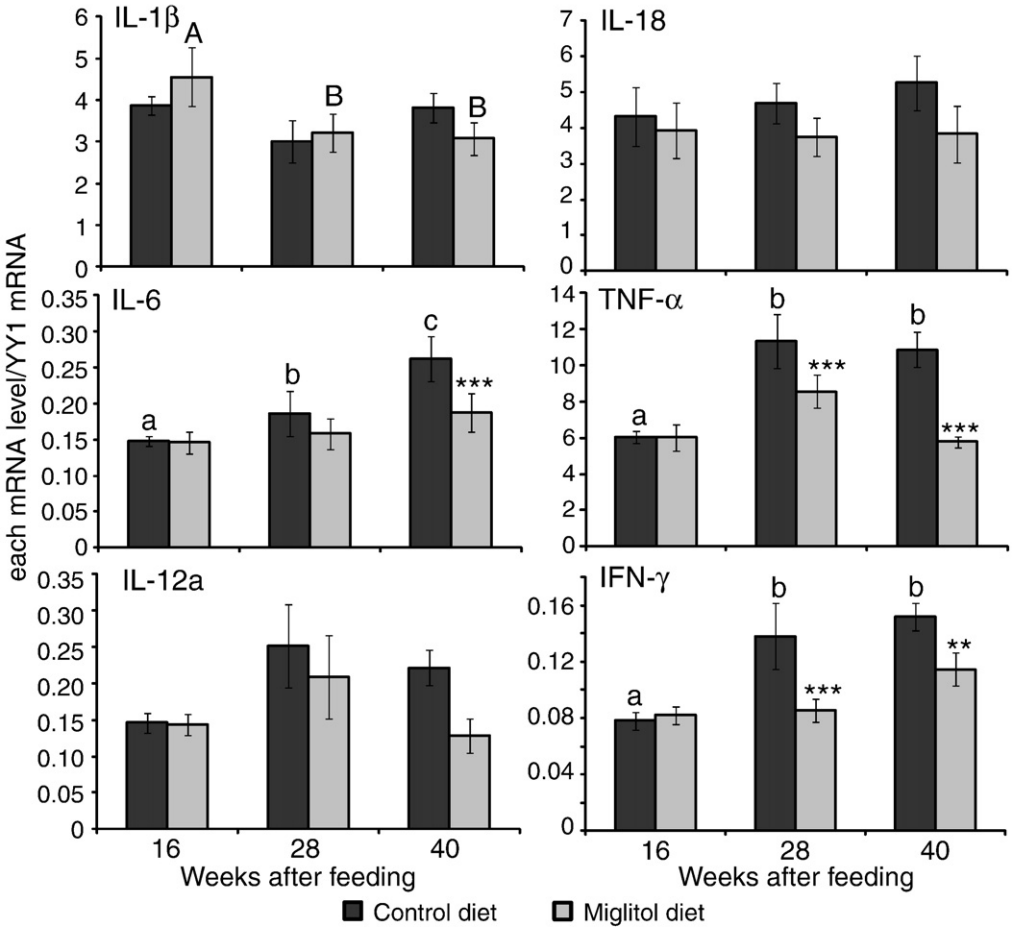

Fig. 2 – Effects of dietary supplementation with miglitol on gene expression of inflammatory cytokines in peripheral leukocytes of OLETF rats. The mRNA levels at weeks 16, 28, and 40 were analyzed by real-time RT-PCR, and the results were normalized for YY1 abundance. Values are means ± SEM (n = 7). **P < .01, ***P < .001 vs the control group. Different letters (*a-c* or *A-B*) denote significant differences (*P* <0.05) over time in each group.

control and miglitol-treated rats at weeks 16, 28, and 40. In control rats, the expression of IL-6, TNF-α, and IFN-γ in peripheral leukocytes gradually increased during the experiment, with significant increases in IL-6 at week 40, TNF-α at weeks 28 and 40, and IFN-γ at weeks 28 and 40, as compared with their expressions at week 16. These increases were not observed in miglitol-treated rats. Interleukin-6 at week 40, TNF-α at weeks 28 and 40, and IFN-γ at weeks 28 and 40 were significantly lower in miglitol-treated rats than in control rats (Fig. 2).

4. Discussion

In this study, we investigated whether treatment with miglitol from the preonset stage improves glycemic control in OLETF

Table 3 – Effect of miglitol on body weight, food intake, and blood glucose in Otsuka Long-Evans Tokushima Fatty (OLETF) rats

	Test substance	Week 0	Week 16	Week 28	Week 40
Body weight (g)	Control	169 ± 6	664 ± 19	758 ± 33	889 ± 63
	Miglitol	164 ± 5	608 ± 23	738 ± 29	869 ± 37
Food intake (g)	Control	–	20.9 ± 0.6	20.8 ± 0.6	26.6 ± 1.4
	Miglitol	–	21.2 ± 1.0	22.0 ± 0.8	24.7 ± 2.4
1,5-AG	Control	–	–	–	11.9 ± 3.4
	Miglitol	–	–	–	21.1 ± 1.2[a]
Triacylglycerol	Control	–	–	–	203 ± 16
	Miglitol	–	–	–	209 ± 23

Values are expressed as means ± SEM for 7 animals.
[a] Significant difference compared with OLETF rats fed a control diet by Student *t* test.

rats. As shown in Fig. 1, the nonfasting blood glucose concentration gradually increased in OLETF rats fed the control diet during the experimental periods, but not in those treated with miglitol, which is consistent with our previous study [22]. Furthermore, dietary supplementation with miglitol for 40 weeks increased 1,5-AG levels. 1,5-AG is a monosaccharide, the plasma concentration of which decreases during periods of sustained or postprandial increases in blood glucose levels through competitive inhibition of glucose reabsorption in the kidney [23]. This indicates that 1,5-AG is a marker for glucose fluctuations. Collectively, these results indicate that treating OLETF rats from the preonset stage with miglitol improves glucose fluctuations and inhibits the development of diabetes.

In this study, we determined the mRNA levels of inflammatory cytokines in peripheral leukocytes obtained from OLETF rats because our previous studies have demonstrated that these mRNA levels respond more rapidly to the progression of diabetes than do the corresponding plasma protein levels [17,24,25]. Interestingly, the mRNA expression of IL-6, TNF-α, and IFN-γ in peripheral leukocytes increased gradually in OLETF rats fed the control diet with the development of diabetes, but not in those fed the miglitol diet. Furthermore, supplementation with miglitol reduced the mRNA levels of IL-6 at week 40, TNF-α at weeks 28 and 40, and IFN-γ at weeks 28 and 40 (Fig. 2). This is the first report showing that the expression of these inflammatory cytokine genes in peripheral leukocytes gradually increased during the development of diabetes from the preonset stage and that miglitol inhibited these changes. Our results in this study suggest that the mRNA levels of inflammatory cytokines in peripheral leukocytes are suitable biomarkers to assess improvements in glucose fluctuations in the early stages of diabetes. Our previous studies have revealed that miglitol supplementation for 4 weeks reduced the expression of inflammatory cytokines such as IL-1β and TNF-α in peripheral leukocytes obtained from streptozotocin-induced diabetic rats [17]. It should be noted that, in this study, the expression of IL-1β was not reduced by miglitol, whereas the gene expression of IL-6 and IFN-γ was deceased by miglitol. The reason for these differences between the present study and our earlier studies may be due to the characteristics of the animal models, stage of diabetes, diet composition, and duration of treatment. Although it should be examined in detail whether the expression of inflammatory cytokine genes in peripheral leukocytes varies between different stages of diabetes and the preonset stage, the results of these studies indicate that the changes in expression of these genes are closely associated with the development, progression, and improvements in diabetes and its complications. Future studies should examine whether the expression of these genes in peripheral leukocytes in humans is associated with inflammation, development, and progression of diabetes and its complications.

It should be noted that inflammatory cytokines are highly expressed in adipose tissue macrophages. Previous studies have demonstrated that treatment with rosiglitazone, a thiazolidine derivative, improved glycemic status and reduced TNF-α mRNA levels in visceral fat in OLETF rats [26]. Thus, miglitol treatment to OLETF rats may reduce the mRNA levels of inflammatory cytokines in adipose tissues. Accordingly, studies should examine whether miglitol affects the mRNA levels of inflammatory cytokines in adipose tissues from OLETF rats.

The mechanism by which miglitol reduces the expression of these inflammatory cytokines in peripheral leukocytes is still unknown. Previous studies have demonstrated that exposure to high glucose upregulates the mRNA expression of TNF-α and IL-1β in monocytic cells [27,28]. It was also reported that the induction of TNF-α mRNA expression in monocytic cells treated with high glucose was due to the activation of nuclear factor–κB caused by the production of reactive oxidative species. Thus, the reduced expression of inflammatory cytokines in peripheral leukocytes from miglitol-treated OLETF rats may be due to reduced nuclear factor–κB activity and reactive oxidative species production; and studies are needed to confirm this concept.

It was reported in the epidemiological Study to Prevent Non–Insulin-Dependent Diabetes Mellitus that inhibition of postprandial hyperglycemia by an α-glucosidase inhibitor from the preonset stage reduced the incidence of diabetes and its complications [1,14,15,29]. In this study, we revealed that treatment with the α-glucosidase inhibitor miglitol in an animal model inhibits the development of diabetes and reduced the gene expression of inflammatory cytokines in peripheral leukocytes. It seems likely that miglitol inhibits the development of diabetes, at least in part, by reducing the expression of inflammatory cytokines in peripheral leukocytes. Nevertheless, further studies are needed to examine whether the inhibition of glucose fluctuation suppressed inflammatory cytokine gene expression in peripheral leukocytes.

In conclusion, the results in this study suggest that treatment with miglitol from the preonset stage in OLETF rats improves glycemic control and reduces the expression of genes related to inflammation in peripheral leukocytes of OLETF rats.

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