Combined Effects of a Dietary Fiber Mixture and Wheat Albumin in a Rat Model of Type 2 Diabetes Mellitus

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Summary As bioactive ingredients of functional foods, dietary fiber and wheat albumin (WA) are known to suppress hyperglycemia in patients with type 2 diabetes mellitus. The combined effects of these bioactive ingredients were examined using an animal model of type 2 diabetes mellitus. First, oral starch tolerance tests (OSTTs) with the simultaneous intake of a dietary fiber mixture (DF) and WA were performed as an acute study. Male Goto-Kakizaki rats received a soluble starch solution [700 mg/kg body weight (bw)] containing DF and/or WA (each 300 mg/kg bw). In these OSTTs, the combined intake of DF and WA suppressed hyperglycemia much more effectively than each separate intake. Second, in a chronic intake study, diets containing DF and/or WA were administered to male Zucker diabetic fatty rats over 84 d. The combined effects of DF and WA were not observed in glycosylated hemoglobin concentration levels or fasting blood glucose levels, but appeared as an improvement in liver lipid contents. Variations in the liver lipid contents were similarly reflected in those of the plasma lipid concentrations. In conclusion, this study found that the simultaneous intake of bioactive DF and WA improved the postprandial hyperglycemia and the chronic lipid metabolism disorders in rat models of type 2 diabetes mellitus.

Key Words type 2 diabetes mellitus, dietary fiber, wheat albumin, combined effect, lipid metabolism disorder

In type 2 diabetes mellitus, postprandial hyperglycemia caused by a lack of prompt insulin secretion is closely associated with insulin resistance and thus hyperglycemia, which leads to a lipid metabolism disorder that results in arteriosclerosis (1–3). Some dietary fiber, such as water-soluble dietary fiber, physically retards the absorption of glucide and suppresses postprandial hyperglycemia (4). In Japan, therefore, the dietary fibers extracted mostly from various plants are used as functional ingredients of foods. On the other hand, wheat albumin (WA), based on the action of its principle component 0.19-albumin (termed by its electrophoretic mobility), inhibits mammalian amylase (5, 6), delays glucide digestion *in vivo*, and suppresses postprandial hyperglycemia (7). Thus, WA is widely distributed as a bioactive ingredient of food for specified health uses (FOSHU) in the market. Various consumers, intentionally or unintentionally take simultaneously more than two of these functional ingredients in foods. However, the combined effects of dietary fiber and WA on the postprandial hyperglycemia and also on chronic glucide and lipid metabolism are unknown. Here the simultaneous intake of these ingredients was examined using a rat model of type 2 diabetes mellitus.

MATERIALS AND METHODS

Approval and feeding conditions. All rats were maintained under standard conditions of $23 \pm 2^\circ\text{C}$ and

$55 \pm 15\%$ humidity with a 12 h/12 h light/dark cycle. The rats were individually housed in clean cages with free access to water and basal diet (CLEA rodent diet CE-2; CLEA Japan, Inc., Tokyo, Japan) *ad libitum* during the acclimation period. All the animal studies were performed at Gifu University and were approved by the Animal Care and Use Committee for Animal Experimentation of Gifu University (Acute study, approval number: 24-86; Chronic study, approval number: 25-17).

Bioactive ingredients. The dietary fiber mixture (DF) was provided by Miki Corporation (Osaka, Japan). The raw materials of DF were as follows: oat bran, chicory root extract, guar bean split-product, young barley leaves, oat fiber, konjac potato extract, green oat extract, and seaweed extracts. The total dietary fiber content of DF was 54.4%, which included 39.9% water-soluble dietary fiber, and 14.5% water-insoluble dietary fiber.

WA is a bioactive ingredient of food for FOSHU and provided by Miki Corporation. WA, which is a water-soluble protein fraction of wheat, is known to inhibit mammalian salivary and pancreatic amylases (8).

Acute study. Animals and experimental design: The oral starch tolerance tests (OSTTs) were performed as a randomized, four-way, cross-over trial. Eight-week-old male Goto-Kakizaki (GK) rats ($n=12$) were purchased from Japan SLC, Inc. (Shizuoka, Japan). After a 14-d period of acclimatization with basal diet, OSTTs were performed at four measurement points: 10, 11, 12, and 13 wk old. A wash-out period of 1 wk was maintained between each OSTT. Since there was no statistically sig-

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Table 1. Composition of experimental diets given to ZDF rats (g/100 g diet).¹⁻⁴

Group	Lean (reference)	Fatty			
		Control	DF	WA	DF+WA
DF	0.00		10.00	0.00	10.00
WA	0.00		0.00	5.00	5.00
Cornstarch	39.75		34.75	39.75	34.75
α -Cornstarch	23.20		23.20	23.20	23.20
Milk casein	20.00		20.00	15.00	15.00
Purified soybean oil	7.00		7.00	7.00	7.00
Cellulose powder	5.00		0.00	5.00	0.00
Mineral mixture (AIN-93G-MX)	3.50		3.50	3.50	3.50
Vitamin mixture (AIN-93VX)	1.00		1.00	1.00	1.00
L-Cystine	0.30		0.30	0.30	0.30
Choline bitartrate	0.25		0.25	0.25	0.25
<i>tert</i> -Butylhydroquinone	0.0014		0.0014	0.0014	0.0014

¹ Basic components were formulated on the basis of the AIN-93G (9). DF cellulose powder replaced cornstarch and cellulose power, and WA was substituted for milk casein.

² Protein derived N contents of WA and milk casein were 14.8 and 13.5%, and those of the diets of control, DF, WA, and DF+WA were 13.5, 13.5, 13.8, and 13.8%, respectively.

³ The energy density of the diets of control, DF, WA, and DF+WA was 3.7, 3.7, 3.7, and 3.8 kcal/g, respectively.

⁴ DF, dietary fiber mixture; WA, wheat albumin.

nificant difference among the values of fasting blood glucose measured at these four times ($p > 0.05$, Tukey's test), adoption of the randomized, four-way, cross-over trial was judged appropriate.

After 15 h of fasting, the rats received soluble starch solution [700 mg/kg body weight (bw)] with bioactive ingredients, DF (300 mg/kg bw), WA (300 mg/kg bw), or DF+WA (300 mg/kg bw + 300 mg/kg bw). Blood from the tail vein was collected into capillary tubes containing lithium-heparin 0, 30, 60, 120, and 180 min after receiving the test solutions. Blood samples were centrifuged ($15,000 \times g$ for 15 min) to separate the plasma.

Blood glucose: The concentration of glucose in the plasma was measured using a commercial enzymatic assay kit (Glucose CII-Test Wako, Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Chronic study. Animals and diet: Five-wk-old male Zucker diabetic fatty (ZDF) rats ($n=24$), as well as lean rats ($n=6$) for use as the reference, were purchased from Charles River Laboratories Japan, Inc. (Yokohama, Japan). After a 7-d acclimatization period on the basal diet, six rats per group were maintained for 84 d on an experimental diet formulated on the basis of the AIN-93G diet for rodents (9). DF was substituted for cornstarch and cellulose powder, and WA was substituted for milk casein (Table 1). Food and water were available ad libitum.

Fasting blood glucose: Fasting blood glucose was measured 5, 10, and 12 wk after the start of the chronic study. After an overnight fast, the blood glucose measurement was performed using the method described above for the acute study.

Treatment of tissues: After 12 wk, the (18-wk-old) rats were sacrificed by cardiac puncture after an overnight fast. Liver, perirenal fat, and epididymal fat were resected and weighed immediately. Part of the liver was immediately stored at -80°C until needed for analysis.

The remainder was immediately stored in the RNA stabilization solution RNeasy (Ambion, Austin, TX, USA), which preserved the RNA in the tissue before storage at -80°C for total RNA extraction. Plasma was fractionated from a part of the blood by centrifugal separation.

Assay procedures: Total liver lipids were extracted by the method of Bligh and Dyer (10). Briefly, appropriate volumes of chloroform and methanol (1 : 2) were added to the tissue (0.5 g) homogenized in 1 mL of distilled water in order to obtain a one-phase system consisting of $\text{CHCl}_3/\text{CH}_3\text{OH}/\text{H}_2\text{O}$ (1 : 2 : 0.8, v/v/v). After vigorous mixing, the appropriate volumes of chloroform and water were added to obtain a two-phase system $\text{CHCl}_3/\text{CH}_3\text{OH}/\text{H}_2\text{O}$ at 1 : 1 : 0.9 ratios (v/v/v) followed by another vigorous mixing. Then, the mixture was centrifuged to allow the formation of the two liquid phases. The chloroform phase containing total lipids was removed and evaporated under nitrogen stream and lipid extract was reconstituted in 2 mL isopropanol. The levels of triglycerides and total cholesterol were measured by using commercial enzymatic assay kits (Wako Pure Chemical Industries, Ltd.), Triglyceride E-test Wako and Cholesterol E-test Wako, respectively.

Glycosylated hemoglobin (HbA1c) concentrations were determined with a high-performance liquid chromatography method performed using an HLC-723 GHbV automatic glycohemoglobin analyzer (Toso, Tokyo) equipped with a boronic acid column (11). Commercial enzymatic assay kits (Wako Pure Chemical Industries, Ltd.) were used to measure the levels of glucose (Glucose CII-test Wako), triglycerides (TG; Triglyceride E-test Wako), total cholesterol (T-Chol; Cholesterol E-test Wako), high density lipoprotein cholesterol (HDL-C; HDL-Cholesterol E-test Wako), and nonesterified fatty acids (NEFA; NEFA C-test Wako) in the plasma.

Analysis of mRNA levels: Total RNA was extracted from liver samples using the mirVana™ PARIS™ kit (Life Technologies, Carlsbad, CA, USA) according to the man-

Table 2. Primers designed for real-time PCR.¹

Gene names	Aliases	Gene ID	Forward primer	Reverse primer
			Sequence 5'→3'	Sequence 5'→3'
<i>Fasn</i>		50671	AGGCTGAAGCTGAAGCCATA	CCAGGCTCTTCAGTGGTAGC
<i>Acaca</i>	ACC1	60581	GGTAGCTGTGCAGTTTGCTG	GCCTCAGTCGCCAGTAGAAG
<i>Mixipl</i>	ChREBP	171078	AAAGCAACCACGCTTCAGA	GGCAGCATTGAGCTCCTCTA
<i>Srebf1</i>	SREBP-1c	78968	CCAACAGTCAGCAGCTTCA	TCAGGTCATGTTGGAAACCA
<i>Hmgcr</i>	3H3M	25675	AGCTTGCTGTGAGAACGTGA	CCATTGGCACCTGGTACTCT
<i>Cyp7a1</i>	CYP7S1	25428	CCCAGACCCCTTTGACTTTCA	GATCCGAAGGGCATGTAGAA
<i>Acadl</i>	LCAD	25287	AGGCTTCTACTACCTCATGCAA	CGTAGTTCTGGTTTCTTCAAA
<i>Acadvl</i>	VLCAD	25363	ATGGCTTCAAGGTTGCTGTC	GAATTTTGTCCCCAAACTGG
<i>Cpt1a</i>	CPT-1a	25757	CTTGTTGAGAAGCACCAGCAC	CCTTCAGGAAAAGGTGAGTCG
<i>Acox1</i>	ACOA1	50681	ATTGCCACTACGTGGTCGTT	GACACAGGTTCTCAGCACA
<i>G6pc</i>	G6Pase	25634	TTGCCAGCCTCTTCAAAAAC	GCAGTTCTCCTTTGCAGCTC
<i>Pck1</i>	PEPCK	362282	ATACGGTGGGAACACTACTGC	GTTATGCCCAGGATCAGCAT
<i>Ppara</i>	PPAR-alpha	25747	TCCCTTTTGTGGCTGCTAT	AATCCCTCCTGCAACTTCT
<i>Pparg</i>	PPAR-gamma	25664	AACGTGAAGCCCATCGAG	CATCTTCTGGAGCACCTTGG
<i>Tnf</i>	TNF-alpha	24835	CAATGGCATGGATCTCAAAG	GTGGGTGAGGAGCAGTAGT
<i>Ccl2</i>	MCP-1	24770	TAGCATCCAGTGCTGTCTC	CCGACTCATTGGGATCATCT
<i>Rn18s</i>		100861533	TTCCGATAACGAACGAGACTCT	TGGCTGAACGCCACTTGTCT

¹ *Acaca*, acetyl coA carboxylase alpha; *Acadl*, acyl-CoA dehydrogenase, long chain; *Acadvl*, acyl-CoA dehydrogenase, very long chain; *Acox1*, acyl-CoA oxidase 1; *Ccl2*, C-C motif chemokine ligand 2; *Cpt1a*, carnitin palmitoiltransferase 1a; *Cyp7a1*, cytochrome P450, family 7, subfamily a, polypeptide 1; *Fasn*, fatty acid synthase; *G6pc*, glucose-6-phosphatase, catalytic subunit; *Hmgcr*, 3-hydroxy-3-methylglutaryl coenzyme A reductase; *Mixipl*, MLX interacting protein-like; ns, not significant ($p>0.05$); *Pck1*, phosphoenolpyruvate carboxykinase 1; *Ppara*, peroxisome proliferator-activate receptor alpha; *Pparg*, peroxisome proliferator-activate receptor gamma; *Rn18s*, 18S ribosomal RNA; *Srebf1*, sterol regulatory element binding transcription factor 1; *Tnf*, tumor necrosis factor.

ufacturer's instructions, before being stored at -80°C . The extracted RNA was reverse-transcribed using a First Strand cDNA Synthesis kit for real-time RT-PCR and the PrimeScriptTM RT reagent Kit (Perfect Real Time; Takara Bio Inc., Shiga, Japan) according to the manufacturer's instructions.

Primers for real-time PCR were designed from the published sequences of rat fatty acid synthase (*Fasn*), acetyl coenzyme A carboxylase alpha (*Acaca*), MLX interacting protein-like (*Mixipl*), sterol regulatory element binding transcription factor 1 (*Srebf1*), 3-hydroxy-3-methylglutaryl coenzyme A reductase (*Hmgcr*), cytochrome P450, family 7, subfamily a, polypeptide 1 (*Cyp7a1*), acyl-CoA dehydrogenase, long chain (*Acadl*), acyl-CoA dehydrogenase, very long chain (*Acadvl*), carnitine palmitoyltransferase 1a (*Cpt1a*), acyl-coenzyme A oxidase 1 (*Acox1*), glucose-6-phosphatase (*G6pc*), phosphoenolpyruvate carboxykinase 1 (*Pck1*), peroxisome proliferator activate receptor alpha (*Ppara*), peroxisome proliferator activate receptor gamma (*Pparg*), tumor necrosis factor (*Tnf*), C-C motif chemokine ligand 2 (*Ccl2*), and 18S ribosomal RNA (*Rn18s*) cDNA (DDBJ/EMBL/GenBank). All primer sequences are shown in Table 2.

Real-time RT-PCR was performed using the StepOne real time PCR system (Thermo Fisher Scientific, Waltham, MA) with a commercially available master mix (Fast SYBR[®] Green Master Mix; Thermo Fisher Scientific) containing Taq DNA polymerase and SYBR-Green I dye. After the addition of the primers and template DNA to the master mix, DNA polymerase activation (95°C for 20 s), 40 cycles of denaturation (95°C for 3 s), and annealing/extension (60°C for 30 s) were performed. Each mRNA level was expressed as the value

relative to the levels of *Rn18s* mRNA.

Statistical analysis. All data were expressed as the mean $\pm$ standard error (SE). Data were analyzed by a two-way ANOVA in order to investigate the combined effects of DF and WA. When an interaction (DF $\times$ WA) was detected to be statistically significant, differences among the groups were tested by Dunnett's multiple range test as a post hoc test.

RESULTS

Acute study

OSTTs. Blood glucose levels after soluble starch load were markedly elevated in GK rats, particularly in rats that were administered the vehicle control, and the glucose peak was observed at 60 min (Fig. 1). At 30 and 60 min during the OSTT, the blood glucose levels of both rats being loaded with DF and WA were lower than those of the rats being loaded with vehicle. The effects of each of these bioactive ingredients were confirmed as their main effect (Table 3). Furthermore, at 60 min during the OSTT, the blood glucose levels of rats that were simultaneously administered DF and WA were lower than those of rats that were separately administered these bioactive ingredients. The suppressive effect of the blood glucose peak by the combination was confirmed as the interaction of DF and WA (Table 3).

Chronic study

Food intake, body weight, and tissue weight. Food intake, initial body weight, and tissue weights were higher in the control group than in the lean group (Table 4). The food intake tended to be low in the WA group and DF+WA group, relative to that of the control group, and the main effect of WA was detected. On the

Fig. 1. Plasma glucose levels before (0 min) and during (30, 60, 120 and 180 min) OSTTs with DF and/or WA. $n=12$. The differences among the groups were tested by Dunnett's multiple range test as a post hoc test (** $p<0.01$) only when an interaction (DF $\times$ WA) were detected by two-way ANOVA.

Table 3. Main effect and interaction of DF and WA in variations of blood glucose levels in OSTTs.

min	Statistical significance, p		
	DF main effect	WA main effect	DF $\times$ WA interaction
30	<0.001	<0.001	0.06 (trend)
60	<0.001	<0.001	0.003
120	0.07 (trend)	<0.001	ns
180	ns	ns	ns

p value less than 0.05 was shown after rounding off and $p<0.10$ was considered to indicate a trend (two-way ANOVA). ns, not significant ($p>0.10$).

other hand, the liver weights were low in the DF group compared to that in the control group, and the main effect of the DF was detected. On the other hand, the perirenal fat weight (g/100 g bw) decreased in the WA and DF+WA groups from that in the control group, and the main effect of WA was detected.

Fasting plasma glucose level and HbA1c concentration. The fasting blood glucose and HbA1c levels were high in the control group relative to those of the lean group (Table 5). HbA1c levels tended to be low in the WA and DF+WA groups, relative to that in the control group, and the main effect of WA was observed.

Plasma and liver lipid levels. All the plasma lipid levels were higher in the control group than in the lean group (Table 6). The plasma TG, T-Cho, HDL-C, and NEFA levels of the DF and DF+WA groups were lower than those of the control group, and the main effects of DF were detected, respectively.

The liver TG and T-Cho levels were higher in the control group than in the lean group, and those in rats fed the bioactive ingredients, particularly DF, were significantly lower than those in the control group. Therefore, the main effects of DF and an interaction were detected in the liver TG and T-Cho levels, respectively.

Table 4. Food intake, body weight, weight gain, and tissue weights in ZDF rats maintained with DF and/or WA diets for 12 wk.^{1,2}

Measurement items	Lean (reference)		Fatty			Statistical significance, <i>p</i>		
	Control	DF	WA	DF+WA	DF main effect	WA main effect	DF×WA interaction	
Food intake (g/d)	15.0±0.22	31.6±0.63	28.5±0.91	29.9±0.90	ns	0.03	ns	
Initial body weight (g)	130±0.93	158±1.96	157±2.13	158±1.88	ns	ns	ns	
Final body weight (g)	363±5.70	383±10.6	384±5.38	370±16.1	ns	ns	ns	
Body weight gain (g/d)	2.77±0.06	2.68±0.13	2.70±0.07	2.55±0.19	ns	ns	ns	
Liver weight (g)	10.2±0.33	21.1±0.97	20.6±0.60	17.8±0.71	<0.001	ns	ns	
Liver weight (g/100 g bw)	2.80±0.06	5.51±0.12	5.36±0.15	4.81±0.08	<0.001	ns	ns	
Perirenal fat weight (g)	4.70±0.219	15.3±0.74	13.9±0.70	13.6±1.24	ns	ns	ns	
Perirenal fat weight (g/100 g bw)	1.30±0.04	3.98±0.12	3.44±0.13	3.65±0.20	ns	0.04	ns	
Epididymal fat weight (g)	4.24±0.19	8.91±0.37	8.43±0.42	8.31±0.40	ns	ns	ns	
Epididymal fat weight (g/100 g bw)	1.17±0.03	2.33±0.06	2.19±0.08	2.25±0.08	ns	ns	ns	

¹ Values are expressed as mean $\pm$ standard error ($n=6$). To assess the combined effects of DF and WA, two-way ANOVA was employed.

² DF, dietary fiber mixture; ns, not significant ($p>0.05$); WA, wheat albumin.

Table 5. Fasting plasma glucose levels and HbA1c levels in ZDF rats maintained with DF and/or WA diets for 5 and 12 wk.^{1,2}

Measurement items	Lean (reference)			Fatty			Statistical significance, <i>p</i>		
	Control	DF	WA	DF+WA	DF main effect	WA main effect	DF×WA interaction		
Plasma glucose (mg/dL), 5 wk	298±36.1	329±32.3	306±19.4	311±13.7	ns	ns	ns		
Plasma glucose (mg/dL), 12 wk	489±6.74	489±13.9	510±7.02	489±11.7	ns	ns	ns		
HbA1c (%), 12 wk	10.8±0.27	10.8±0.14	10.2±0.23	10.1±0.23	ns	0.01	ns		

¹ Values are expressed as mean ± standard error (*n*=6). To assess the combined effects of DF and WA, two-way ANOVA was employed.

² Abbreviations used: DF, dietary fiber mixture; ns, not significant (*p*>0.05); WA, wheat albumin.

Table 6. Lipid concentrations in plasma and liver in ZDF rats maintained with DF and/or WA diets for 12 wk.^{1,2}

Measurement items	Lean (reference)			Fatty			Statistical significance, <i>p</i>		
	Control	DF	WA	DF+WA	DF main effect	WA main effect	DF×WA interaction		
Plasma TG (mg/dL)	692±149	305±70.1	451±78.2	301±70.5	0.01	ns	ns		
Plasma T-Cho (mg/dL)	301±10.2	235±10.1	274±10.1	222±8.77	<0.001	ns	ns		
Plasma HDL-C (mg/dL)	54.0±1.07	49.8±0.86	50.5±1.26	47.7±1.23	0.002	ns	ns		
Plasma NEFA (μEq/dL)	61.6±6.78	52.2±3.53	69.0±5.21	53.8±3.05	0.02	ns	ns		
Liver TG (mg/g tissue)	7.59±1.41	2.63±0.57**	4.38±0.60*	4.21±0.36*	0.006	ns	0.01		
Liver TG (mg/total tissue)	166±39.2	54.3±5.16**	90.9±13.4*	75.7±8.37*	0.01	ns	0.04		
Liver T-Cho (mg/g tissue)	7.22±1.01	2.99±0.74**	4.99±0.35*	4.92±0.10*	0.004	ns	0.005		
Liver T-Cho (mg/total tissue)	157±29.6	61.5±9.45**	103±9.73*	87.4±4.00*	0.004	ns	0.03		

¹ Values are expressed as mean ± standard error (*n*=6). To assess the combined effects of DF and WA, two-way ANOVA was employed. When an interaction (DF×WA) was detected to be statistically significant, differences among the groups were tested by Dunnett's multiple range test as a post hoc test (**p*<0.05, ***p*<0.01 vs. control).

² DF, dietary fiber mixture; HDL-C, high-density lipoprotein cholesterol; NEFA, non-esterified fatty acid; ns, not significant (*p*>0.05); T-Cho, total cholesterol; TG, triglyceride; WA, wheat albumin.

Table 7. Liver mRNA expressions in ZDF rats maintained with DF and/or WA diets for 12 wk.^{1,2}

Gene names	Lean (reference)	Fatty			Statistical significance, <i>p</i>		
	control	DF	WA	DF+WA	DF main effect	WA main effect	DF×WA interaction
<i>Fasn</i>	1.00±0.13	9.56±2.11	7.65±1.28	11.7±1.85	ns	ns	ns
<i>Acaca</i>	1.00±0.05	3.02±0.46	2.48±0.25	3.78±0.42	ns	ns	ns
<i>Mixipl</i>	1.00±0.10	0.87±0.08	0.88±0.02	0.83±0.07	ns	ns	ns
<i>Srebfl</i>	1.00±0.15	3.79±0.53	2.41±0.41	1.88±0.22	0.001	ns	ns
<i>Hmgcr</i>	1.00±0.08	1.35±0.10	1.73±0.15	2.37±0.44	0.01	ns	ns
<i>Cyp7a1</i>	1.00±0.22	1.50±0.24	1.99±0.22	2.16±0.62	ns	ns	ns
<i>Acadl</i>	1.00±0.02	1.96±0.13	1.73±0.15	1.77±0.11	ns	ns	ns
<i>Acadvl</i>	1.00±0.08	1.90±0.17	1.82±0.18	2.04±0.22	ns	ns	ns
<i>Cpt1a</i>	1.00±0.04	2.11±0.31	1.57±0.26	1.14±0.14	ns	0.01	ns
<i>Acox1</i>	1.00±0.03	2.01±0.19	1.78±0.23	1.98±0.19	ns	ns	ns
<i>G6pc</i>	1.00±0.19	1.73±0.31	1.04±0.15	1.49±0.21	0.006	ns	ns
<i>Pck1</i>	1.00±0.08	1.38±0.08	0.99±0.15*	1.13±0.08	ns	ns	0.03
<i>Ppara</i>	1.00±0.09	3.38±0.56	3.26±0.66	3.40±0.65	ns	ns	ns
<i>Pparg</i>	1.00±0.06	0.81±0.06	0.81±0.11	0.97±0.07	ns	ns	ns
<i>Tnf</i>	1.00±0.06	3.81±0.38	3.93±0.91	5.96±0.55	0.04	ns	ns
<i>Ccl2</i>	1.00±0.26	21.3±8.52	8.7±2.16	36.1±18.8	ns	ns	ns

¹ Values are expressed as mean ± standard error (*n*=6). To assess the combined effects of DF and WA, two-way ANOVA was employed. When an interaction (DF×WA) was detected to be statistically significant, differences among the groups were tested by Dunnett's multiple range test as a post hoc test (**p*<0.05 vs. control).

² *Acaca*, acetyl coA carboxylase alpha; *Acadl*, acyl-CoA dehydrogenase, long chain; *Acadvl*, acyl-CoA dehydrogenase, very long chain; *Acox1*, acyl-CoA oxidase 1; *Ccl2*, C-C motif chemokine ligand 2; *Cpt1a*, carnitin palmitoiltransferase 1a; *Cyp7a1*, cytochrome P450, family 7, subfamily a, polypeptide 1; *Fasn*, fatty acid synthase; *G6pc*, glucose-6-phosphatase, catalytic subunit; *Hmgcr*, 3-hydroxy-3-methylglutaryl coenzyme A reductase; *Mixipl*, MLX interacting protein-like; ns, not significant (*p*>0.05); *Pck1*, phosphoenolpyruvate carboxykinase 1; *Ppara*, peroxisome proliferator-activate receptor alpha; *Pparg*, peroxisome proliferator-activate receptor gamma; *Srebfl*, sterol regulatory element binding transcription factor 1; *Tnf*, tumor necrosis factor; WA, wheat albumin.

Gene expression. The gene expression levels in the liver, except for those of the genes encoding *Mlxipl* and *Pparg*, were high in the control rats from those in the lean rats (Table 7). The *Srebf1* and *G6pc* mRNA expression levels were lower in the DF and DF+WA groups than in the control group, and the main effects of DF were detected, respectively. The *Hmgcr* and *Tnf* mRNA expression levels were high in the DF and DF+WA groups compared to those in the control group, and the main effects of DF were detected, respectively. On the other hand, the *Cpt1a* mRNA expression level was low in the WA and DF+WA groups compared to that in the control group, and the main effect of WA was detected. *Pck1* mRNA expression level was significantly lowered in the DF and WA groups, but not in the DF+WA groups, from that in the control group, and an interaction of DF and WA was detected.

DISCUSSION

In this study, the combined effects of DF and WA against hyperglycemia and dyslipidemia were investigated in acute and chronic studies with type 2 diabetes mellitus rats.

In the acute OSTTs, an interaction was observed for the combined intake of DF and WA. The combined effect was thought to be due to each function of DF and WA as follows. Because the DF used for the OSTTs included 14.5% water-soluble dietary fiber, its ability to suppress hyperglycemia when administered alone (Fig. 1) was thought to be due to its ability to inhibit the diffusion of glucides through gel formation by the water-soluble dietary fiber (12). On the other hand, it is clear that WA's ability to suppress hyperglycemia when administered alone (Fig. 1) is due to its ability to inhibit α -amylase activity, since there is an α -amylase inhibitor present in WA (13, 14). In the acute study, the ability of DF to physically inhibit glucide digestion and the ability of WA to inhibit α -amylase activity were additive or partially synergistic and were found to suppress the steep hyperglycemia seen in the type 2 diabetes mellitus rats.

In the chronic ingestion study, the combination of DF and WA had no effect on the HbA1c levels or fasting blood glucose levels. However, WA showed a trend toward lowering the levels of HbA1c, which is an important index of long-term diabetic control (Table 5). WA, which is rich in α -amylase inhibitor, is a bioactive ingredient of FOSHU for which a disease risk reduction is authorized in Japan.

Accordingly, WA was thought to show constant and partly suppressive effects for the severe diabetes mellitus ZDF rats. On the other hand, Scott et al. (15) reported that the effects of dietary fiber were not obtained in poorly controlled humans with more than 10% HbA1c. The HbA1c levels of the ZDF rats in this study were 10% or more. Also, in patients with poor glycemic control and more than 9.3% HbA1c, the contribution ratio of the suppressed postprandial blood glucose level for HbA1c is low; approximately 40% (16). In other words, HbA1c shows a stronger relationship with fasting blood glucose level than with postprandial blood glucose (17,

18). The Diabetes Epidemiology: Collaborative Analysis of Diagnostic Criteria in Europe (DECODE) study demonstrated that cardiovascular mortality correlates with the peak glucose level within 2 h of glucose loading, and the risk of death among diabetic patients cannot be discriminated using only fasting blood sugar data (19). Even if the combined effect of DF and WA was not clear in a chronicity study, if the inhibition of postprandial hyperglycemia is important in diabetes prevention and cardiovascular mortality, the combined effect on postprandial hyperglycemia observed in the acute experiment (Fig. 1) will contribute at least to diabetes prevention.

The combined effect observed upon chronic intake of DF and WA was the improvement of liver lipids (Table 6). In comparison with the ZDF rat and the lean rat, though the final body weight was almost the same, the difference of the liver weight was double (Table 4), and that of the TG weight per total liver resulted in a four-fold increase in the ZDF rat (Table 6). Nevertheless, it is thought that the ZDF rat in this study did not develop a remarkable fatty liver. In ZDF rats, fatty liver was not found until 15 wk of age, but it was observed after 20 wk of age (20). Therefore, at 18 wk of age when the anatomy was examined in this study, the lipid accumulation in the liver of the ZDF rat was mild and it was regarded that the fatty liver was in progress. The initial step of fatty acid biosynthesis is the conversion of acetyl-CoA to malonyl-CoA. Expression of the RNAs encoding *Acaca* and *Fasn*, which are enzymes in the fatty acid biosynthetic pathway, is promoted by *Srebf1* (21, 22). However, the expression of *Acaca* and *Fasn*, which is controlled synchronously by *Srebf1*, showed a lowering trend because *Srebf1* gene expression tended to decrease in the livers of DF-fed rats. On the other hand, the intake of WA suppressed expression of the *Cpt1a* gene and showed a trend toward lowering the levels of liver lipids. That is to say, in the WA intake group, the expression of *Cpt1a*, which catalyzes the rate-determining step of β -oxidation, was thought to decrease along with the decrease in the levels of liver lipids, which are the substrate of β -oxidation. The depression of the *Cpt1a* gene expression in the liver of the WA intake group caused a depression trend of plasma TG concentrations and, through a systemic circulation, might be related with the depression of the perirenal fat weight per body weight. *Hmgcr* (23), which is a rate-limiting key enzyme of the cholesterol biosynthetic pathway, and strongly controls cholesterol synthesis, but its transcription is also controlled by the intracellular cholesterol content; thus, this enzyme maintains internal cholesterol homeostasis (24). Because intracellular cholesterol content decreased conspicuously in the livers of DF-fed rats, *Hmgcr* gene expression was thought to be promoted in the DF intake groups. Expression of the gene encoding *G6pc* tended to be low in the DF group relative to that in the control group. The result suggests that the inhibition of gluconeogenesis and glucose secretion from the liver might be associated with the antidiabetic efficacy of DF. The expression of *Pck1*, which promotes gluconeogenesis, tended to be suppressed in the liver of DF- or WA-fed

rats, but the reason for the offset by the combined intake is not clear. On the other hand, a trend toward increased expression of the *Tnf* gene in the liver was recognized in the DF intake groups. Because *Tnf* induces insulin resistance, a future survey concerning its relationship with the intake of DF is warranted.

The changes in plasma lipid concentrations (TG, T-Cho, and HDL-C) were associated with those of liver lipid contents (Table 6). A conspicuous improvement in liver lipid levels from those in the WA group was observed in the DF group (Table 6). In most cases, diets with increased DF improve hyperlipidemia (25). On the other hand, when refined DF and foods high in DF content are ingested, their effects vary according to the kind of dietary fiber and the food (25). Generally, the lipid-lowering effect is strong in the water-soluble and high viscous dietary fiber, but the effect of water-insoluble dietary fiber is weak or ineffective. Guar gum, pectin, oats and legumes have a particularly remarkable effect. The oat bran-derived β -glucan was the primary component in the DF used in this study. We speculate that the mechanism by which DF ingestion reduces blood cholesterol levels is as follows (26). First, the dietary fiber increases fecal bile acid excretion. The effect may not be caused by promoted catabolism of cholesterol in the liver. In fact, expression of the gene encoding *Cyp7a1*, which is the rate-limiting enzyme of cholesterol catabolism in the liver, did not change (Table 7). In this study, however, anatomy was examined after fasting overnight in consideration of the disappearance of diet-derived chylomicrons. Thus, the possibility that fasting affected the mRNA level cannot be entirely ruled out. On the other hand, dietary fiber is thought to adsorb bile acids. As a result, micellar formation is suppressed and thus, absorption of various lipids in the small intestine is suppressed. This effect is strong especially in highly viscous, water-soluble dietary fiber. The main component of the water-soluble dietary fiber found in the cell wall of barley, oats, and wheat is β -glucan (27). The plasma cholesterol-reducing effect of this substance has been identified in many studies in humans and experimental animals (28–31).

Chicory is the primary raw material for the production of inulin, which is present in this plant at 15–20% wet weight (32, 33). Inulin is thought to inhibit hyperglycemia by promoting the secretion of pancreatic insulin. In other words, inulin inhibits hyperglycemia as follows (34–36). Inulin is assimilated by intestinal flora. The short chain fatty acids produced by these flora stimulate enteroendocrine cells, which, in turn, secrete glucagon-like peptide-1, which promotes pancreatic insulin secretion. Guar gum is composed primarily of galactomannan, a polysaccharide included in the guar seed. Guar gum is a water-soluble polysaccharide known to reduce blood glucose and/or cholesterol levels (37–39). Glucomannan, a high-viscosity soluble dietary fiber, has been shown to improve glycemic control and peripheral insulin sensitivity in type 2 diabetes (40). Konjacmannan, a natural constituent of konjac root, is a highly viscous water-soluble fiber that has been shown to reduce fast-

ing and postprandial glycemia, as well as cardiovascular risk factors (41). Alginate has been reported to have high viscosity and gel organization potency and shown to suppress hyperglycemia, and also to promote fecal bile acid and cholesterol excretion (42). Thus, the improvements in lipid metabolism seen with DF in this study are thought to be the comprehensive effects of these water-soluble dietary fibers.

In conclusion, this study demonstrated that the combined intake of DF and WA improved the postprandial hyperglycemia and the chronic lipid metabolism disorders in an additive or partial synergistic manner in rat models of type 2 diabetes mellitus.

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