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Effects of Wheat Albumin Consumption on Expression of Genes Related to Lipogenesis and Insulin Sensitivity in Adipose Tissues of Rats

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Suppression of postprandial hyperglycemia reduces lipogenic enzyme activities in the adipose tissues of normal rats. The present study investigated the expression of genes related to lipogenesis and insulin sensitivity in mesenteric adipose and epididymal adipose tissues to evaluate the effects of wheat albumin (WA) and a crude preparation of WA (CWA) with α-amylase inhibitory activity on lipid metabolism. Rats fed 2.5% WA, which had 12.7-fold inhibitory activity compared with CWA, exhibited reduced mRNA levels for G6PDH, ACO, ACS, PEPCK, and LPL in the mesenteric adipose, but not in the epididymal adipose tissue. Linear regression analyses showed that the gene expression levels of FAS, G6PDH, ACS, and LPL were reduced in dose-dependent manners in the mesenteric adipose tissue of rats fed the CWA diet. These results suggest that supplementation with CWA as well as WA reduces the expression of genes related to lipogenesis and insulin sensitivity in mesenteric adipose tissue.

KEYWORDS: Hyperglycemia; wheat albumin; lipogenic enzyme; mesenteric adipose tissue

INTRODUCTION

Recently, various food components and drugs that decrease postprandial hyperglycemia/hyperinsulinemia by inhibiting α-amylase from the pancreas or α-glucosidase in the small intestine have been developed. Wheat albumin (WA), which is extracted from wheat, has been reported to act as an inhibitor of pancreatic α-amylase and ameliorates postprandial hyperglycemia/hyperinsulinemia (*1*−*4*). The specific α-amylase-inhibiting proteins in WA have already been identified as 0.19, 0.28, 0.36, and 0.53 fractions by gel electrophoresis (*5*). The 0.19 fraction, the amino acid sequence of which has already been determined (*4*, *5*), was found to exert the predominant α-amylase inhibitory activity in WA (*2*, *3*). WA including these fractions has greater inhibitory effects on α-amylase activity than other types of α-amylase inhibitors, such as white beans (*2*). Thus, it is thought that WA may be useful for patients with postprandial hyperglycemia/hyperinsulinemia, even as a crude preparation.

Many recent studies have indicated that inhibition of postprandial hyperglycemia/hyperinsulinemia in normal animal models reduces lipogenesis in white adipose tissues as well as plasma triacylglycerol (*6*−*10*). Indeed, our recent study demonstrated that feeding normal model rats a diet containing high-amylose corn starch, which caused a lower postprandial glucose level than general corn starch due to its slower rate of digestion, reduced the activities of lipogenic enzymes in their mesenteric adipose tissue (*11*). It is believed that many food components with α-amylase inhibitory activities have similar effects on lipogenic enzymes in adipose tissues. However, no studies have investigated whether inhibition of α-amylase by a crude preparation of WA (CWA), as well as WA, reduces the expression of genes related to lipogenesis in white adipose tissues.

In the present study, we examined whether dietary supplementation with WA and CWA could alter the expression of genes related to lipogenesis and insulin sensitivity in the white adipose tissues of normal rats. In addition, we determined the effects of CWA/WA on the expression of these genes in mesenteric adipose tissue, which is referred to as visceral adipose tissue in humans, and in another adipose tissue depot, epididymal adipose tissue, to evaluate whether the response to CWA/WA of visceral adipose tissue is different from that of other adipose tissues.

MATERIALS AND METHODS

Preparation of WA as an Inhibitor for α-Amylase. WA was prepared as previously described (*3*). For CWA, supernatants obtained by centrifuging wheat extracts dissolved in deionized water were heated (80 °C, 30 min). The solutions were then centrifuged again, and the

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Table 1. Composition of Wheat Albumin

	CWA (%)	WA (%)
water	4.90	6.25
protein	37.00	80.30
fat	0.20	0.50
fiber	19.60	
ash	3.00	4.65
carbohydrate	35.30	8.30
total	100	100

Table 2. Composition (Grams per Kilogram) of Diets

		CWA			WA
	control	2.5%	5%	10%	2.5%
casein	150	150	150	150	150
α-cornstarch	500	500	500	500	500
corn oil	50	50	50	50	50
lard	100	100	100	100	100
cellulose	50	50	50	50	50
AIN^{93} mineral mix	35	35	35	35	35
AIN^{93} vitamin mix	10	10	10	10	10
choline bitartrate	3	3	3	3	3
L-cystine	2	2	2	2	2
WA					25
CWA		25	50	100	
inactivated CWA	100	75	50		75
total	1000	1000	1000	1000	1000

supernatants were spray-dried. The resulting powder was used as CWA. The composition of materials is shown in **Table 1**. WA contained 29.4% of the 0.19 fraction of WA, whereas CWA contained 1.5%, as determined by HPLC, as described previously (*3*). Both materials were provided by Nisshin Pharma Inc. (Saitama, Japan).

α-Amylase Inhibitory Activity Assay. Various concentrations of WA and CWA were incubated with 37 units/mL human pancreatic α-amylase, 0.13% bovine serum albumin (BSA), and 266 μg/mL soluble starch in 0.25 M sodium phosphate buffer at 37 °C for 7.5 min and analyzed for their α-amylase activities using the Amylase Test Wako (Wako Chemicals, Tokyo, Japan). The results for each sample were expressed as the mean value relative to that of α-amylase without WA (set as 1).

Animals. Six-week-old male Wistar rats were purchased from Japan SLC Inc. (Hamamatsu, Japan) and housed in a room controlled for temperature (22 ± 3 °C), humidity (55 ± 15%), and light (lights on, 7:00 a.m.−7:00 p.m.).

All animals received a standard laboratory chow (MF; Oriental Yeast Co., Tokyo, Japan) for 2 weeks and then received a preconditioning diet that was similar to the control diet in **Table 2** except that it did not contain WA for 1 week.

At 9 weeks of age, the rats were assigned to five groups on the basis of their body weights. Each group consisted of six or seven animals. The animals in each group had free access to a control diet, a diet containing 2.5, 5, or 10% CWA, or a diet containing 2.5% WA for 2 weeks. The variations in the amounts of CWA or WA in the diets were compensated for by adding an inactivated CWA after autoclaving, such that the total amount of CWA and inactivated CWA remained constant (10%). The diet compositions are shown in **Table 2**. The CWA, inactivated CWA, and WA were provided by Nisshin Pharma Inc. as described above. After the rats had been fed the diets containing WA/CWA/inactivated CWA for 2 weeks, all animals were fasted for 6 h before decapitation. Mesenteric adipose and epididymal adipose tissues were excised and weighed. The experimental procedures used in the present study conformed to the guidelines of the Animal Usage Committee of the University of Shizuoka.

Quantitative RT-PCR. Total RNA was extracted by the acidified guanidine thiocyanate method as previously described (*12*). The total RNA samples were stored at −80 °C for subsequent quantitative RT-PCR analyses. The total RNA samples were converted into cDNAs by RT using SuperScript III reverse transcriptase (Invitrogen, Tokyo, Japan) according to the manufacturer's instructions. To quantitatively estimate the mRNA levels of fatty acid synthase (FAS), acyl-CoA oxidase (ACO), acyl-CoA synthase (ACS), malic enzyme (ME), glucose-6-phosphate dehydrogenase (G6PDH), phosphoenolpyruvate carboxykinase (PEPCK), hormone-sensitive lipase (HSL), lipoprotein lipase (LPL), peroxisome proliferator-activated receptor (PPAR) γ, adipocyte fatty acid-binding protein (aP2), and hypoxanthine guanine phosphoribosyl transferase (HPRT), PCR amplification was performed using a Light-Cycler instrument system (Roche, Tokyo, Japan). Real-time PCR reactions were carried out in a total volume of 20 μL containing 400 nM each of gene-specific primers, cDNAs, and SYBR Premix Ex Taq (Takara, Shiga, Japan). The sequences of the PCR primer pairs are shown in **Table 3**. The cycle threshold (CT) values of each gene detected by real-time RT-PCR were converted into signal intensities by the delta-delta method (*13*), which calculates the difference of one CT value as a 2-fold difference between the signal for each gene and the signal for a gene for normalization (HPRT). The formula used was $[2^{(\text{CT of test gene} - \text{CT of HPRT})}]$.

Table 3. Sequences of Oligonucleotide Primers Used for Real-Time RT-PCR in This Study

	sequence
FAS mRNA	5′-GGATGTCAACAAGCCCAAGTA-3′ 5′-TTACAGAGGAGAAGGCCACAA-3′
ME mRNA	5′-GCATTTCGTCTCCTGAACAAG-3′ 5′-TATCCGAAGAGCAGCAAGAAG-3′
G6PDH mRNA	5′-CTGGCGTATCTTCACACCATT-3′ 5′-TTCATCAGCTCATCTGCCTCT-3′
ACO mRNA	5′-TCCAGATAATTGGCACCTACG-3′ 5′-GCCACCACTTAATGGAAGTCA-3′
ACS mRNA	5′-GCTGCTTATGGATGACCTCAA-3′ 5′-TCACTGACGTGTTTGCTTGTC-3′
PEPCK mRNA	5′-CCGAAGGCAAGAAGAAATACC-3′ 5′-CCCACACATTCAACTTTCCAC-3′
PPARγ-1 mRNA	5′-GCTGCAGCGCTAAATTCATCT-3′ 5′-AATGGCATCTCTGTGTCAACC-3′
PPARγ-2 mRNA	5′-ATGGGTGAAACTCTGGGAGAT-3′ 5′-AATGGCATCTCTGTGTCAACC-3′
aP2 mRNA	5′-CCCAGATGACAGGAAAGTGAA-3′ 5′-GCCTTTCATGACACATTCCAC-3
LPL mRNA	5′-TGTCATCGAGAAGATCCGAGT-3′ 5′-CCCGACTTCTTCAGAGACTTGT-3′
HSL mRNA	5′-ATTCGCCATAGACCCAGAGTT-3′ 5′-TCGATCTCCGTGATATTCCAG-3′
β-actin mRNA	5′-ATGATCTTGATCTTCATGGTGCTA-3′ 5′-GTAAAGACCTCTATGCCAACACAGT-3′
HPRT mRNA	5′-CCAGTCAACGGGGGACATAAAA-3′ 5′-CCATTTTGGGGCTGTACTGCTT-3′

Other Assays. Body weight and food intake were measured once every 2−3 days. For analysis of plasma triacylglycerol, blood taken from the tail vein was collected by capillaries containing heparin/lithium and was centrifuged at 1500*g* for 15 min; the supernatant was used as plasma for triacylglycerol measurement. Plasma triacylglycerols were evaluated using an assay kit containing lipoprotein lipase, glycerol-3-phosphate oxidase, and glycerokinase (Triglyceride E-test; Wako Pure Chemical Industries, Osaka, Japan).

Statistics. The results were subjected to one-way analysis of variance followed by Dunn's multiple-range test or unpaired Student's *t* tests

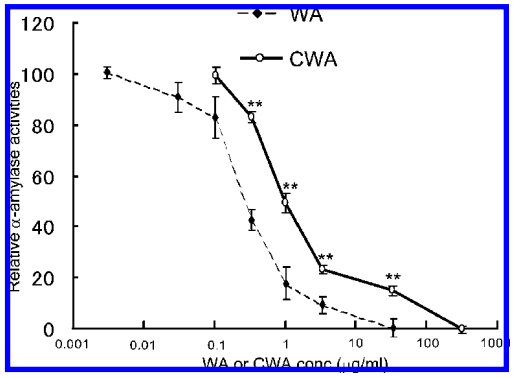

Figure 1. Effects of CWA and WA on pancreatic α-amylase activities in vitro. Various concentrations of CWA and WA were incubated with 37 units/mL pancreatic α-amylase, 0.13% BSA, and 266 μg/mL soluble starch in 0.25 M sodium phosphate buffer at 37 °C for 7.5 min. Each bar represents the mean ± SEM ($n = 4$). Asterisks indicate significant differences compared with levels in WA (**, $p < 0.01$) by unpaired Student's t test.

Table 4. Body Weight and Food Intake of Rats Fed the Diets Containing Various Amounts of CWA or 2.5% WA[a]

	body wt (g)		food intake (g/day)	
test substance	at start	at 2 weeks	at 1 week	at 2 weeks
control	182 ± 4	231 ± 4	11.4 ± 0.4	11.1 ± 0.5
2.5% CWA	189 ± 3	241 ± 6	13.1 ± 0.4	12.3 ± 0.5
5% CWA	189 ± 8	237 ± 9	12.6 ± 0.6	12.1 ± 0.5
10% CWA	190 ± 6	237 ± 7	11.5 ± 0.7	12.2 ± 0.3
2.5% WA	182 ± 3	227 ± 3	11.8 ± 0.2	12.1 ± 0.3

[a] Values are expressed as means ± SEM for five to seven animals.

when appropriate. Linear regression analyses were performed to examine the dose dependencies of the effects of CWA on the expression of genes related to lipogenesis and insulin sensitivity. p values of <0.05 were considered to indicate statistical significance. All statistical analyses were performed using SPSS software version 14.0 for Windows.

RESULTS

Inhibitory Activities of CWA and WA on Pancreatic α-Amylase in Vitro. To evaluate the inhibitory activities of CWA and WA on pancreatic α-amylase, various concentrations of the two preparations were incubated with soluble starch and pancreatic α-amylase. WA at concentrations of 0.1−33 μg/mL had a higher inhibitory activity toward pancreatic α-amylase than CWA at the same concentrations ($p < 0.01$). The IC_{50} values for CWA and WA were 3.55 and 0.28 μg/mL, respectively. Therefore, WA exhibited a 12.7-fold higher inhibitory activity than CWA (**Figure 1**).

Effects of Feeding Rats Diets Supplemented with CWA and WA on Body Weight and Adipose Tissue Weights. The body weight and food intake did not differ among the five groups (**Table 4**). Similarly, the adipose tissue weights did not differ significantly among the five groups (**Table 5**). No adverse signs were observed in any of the groups of rats throughout the experimental period.

Effects of Feeding Rats Diets Supplemented with CWA and WA on Plasma Triacylglycerol Levels. The plasma triacylglycerol concentrations were lower in rats fed the diet containing 2.5% WA than in control rats (**Figure 2**; $p < 0.05$). Linear regression analysis revealed that dietary concentrations of CWA were related to reductions in plasma triacylglycerol ($r = -0.52$; $p < 0.05$).

Table 5. Weight of Liver and Adipose Tissues in Rats Fed the Diets Containing Various Amounts of CWA or 2.5% WA[a]

test substance	mesenteric fat (g)	epididymal fat (g)
control	2.9 ± 0.2	5.0 ± 0.4
2.5% CWA	3.2 ± 0.2	5.4 ± 0.3
5% CWA	3.0 ± 0.3	5.1 ± 0.6
10% CWA	3.2 ± 0.2	5.5 ± 0.4
2.5% WA	2.8 ± 0.2	4.4 ± 0.2

[a] Values are expressed as means ± SEM for five to seven animals.

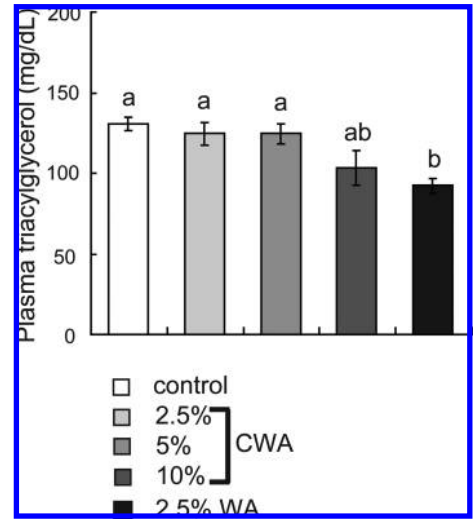

Figure 2. Effects of feeding rats diets containing various concentrations of CWA or 2.5% WA on plasma triacylglycerol levels. Each bar represents the mean ± SEM ($n = 4-6$). Bars not sharing a common letter (a, b) differ significantly at $p < 0.05$ by Dunn's multiple-range test.

Effects of Feeding Rats Diets Supplemented with CWA and WA on the Expression of Genes Related to Lipid Lipogenesis and Insulin Sensitivity in the Adipose Tissues. To determine whether feeding the rats the CWA/WA diets affects lipid metabolism, the expression levels of genes related to lipogenesis and insulin sensitivity were measured in mesenteric and epididymal adipose tissues from rats fed diets containing various concentrations of CWA or 2.5% WA (**Figures 3** and **4**). In comparison to the control diet supplemented with inactivated CWA, animals fed the diet supplemented with 2.5% WA had significantly lower mRNAs levels of G6PDH, ACO, ACS, PEPCK, and LPL ($p < 0.05$). In addition, the diet supplemented with 10% CWA significantly reduced the G6PDH and LPL mRNA levels in the mesenteric adipose tissue (**Figure 3**). Linear regression analyses for groups fed the diets supplemented with various concentrations of CWA revealed that the gene expression levels of FAS ($r = -0.41$, $p < 0.05$), G6PDH ($r = -0.47$, $p < 0.05$), ACS ($r = -0.45$, $p < 0.05$), and LPL ($r = -0.47$, $p < 0.05$) in the mesenteric adipose tissue were reduced in dose-dependent manners. On the other hand, the diets supplemented with various concentrations of CWA preparation or WA did not affect the mRNA levels of the genes examined in the epididymal adipose tissue (**Figure 4**).

DISCUSSION

We found that plasma triacylglycerol concentrations were reduced by feeding rats a diet containing WA, whereas linear regression analysis showed that plasma triacylglycerol levels decreased in a dose-dependent manner according to the concentration of CWA in the diet (**Figure 2**). Our previous study

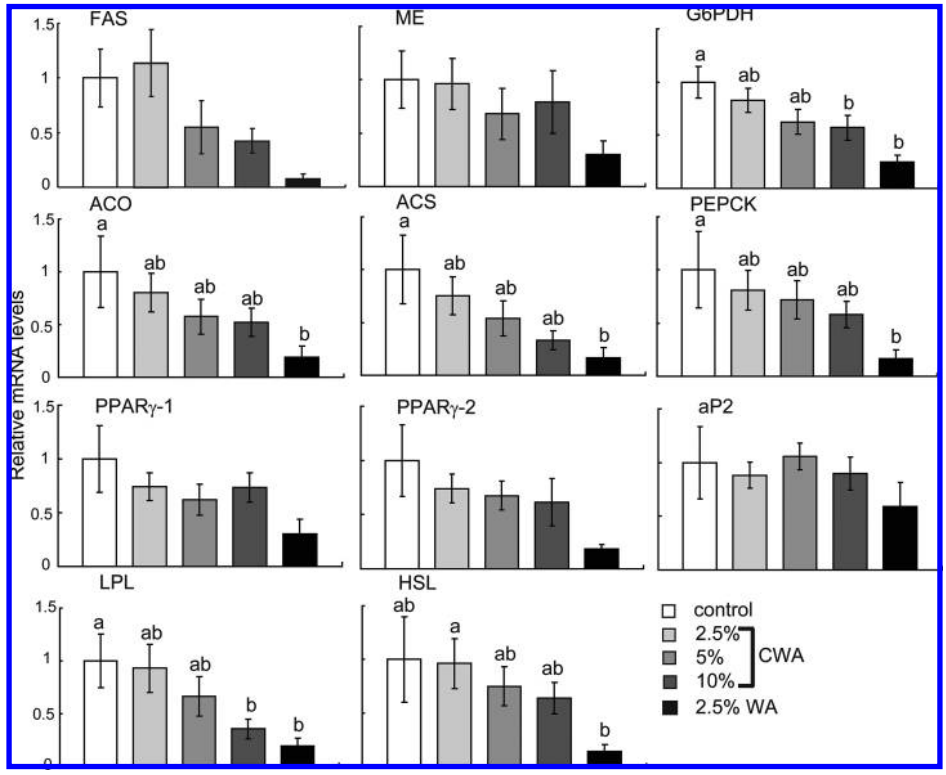

Figure 3. Effects of feeding rats diets supplemented with various concentrations of CWA or 2.5% WA on the expression levels of genes related to lipid metabolism and insulin sensitivity in mesenteric adipose tissue. Each bar represents the mean ± SEM ($n = 5-7$). Bars not sharing a common letter (a, b) differ significantly at $p < 0.05$ by Dunn's multiple-range test.

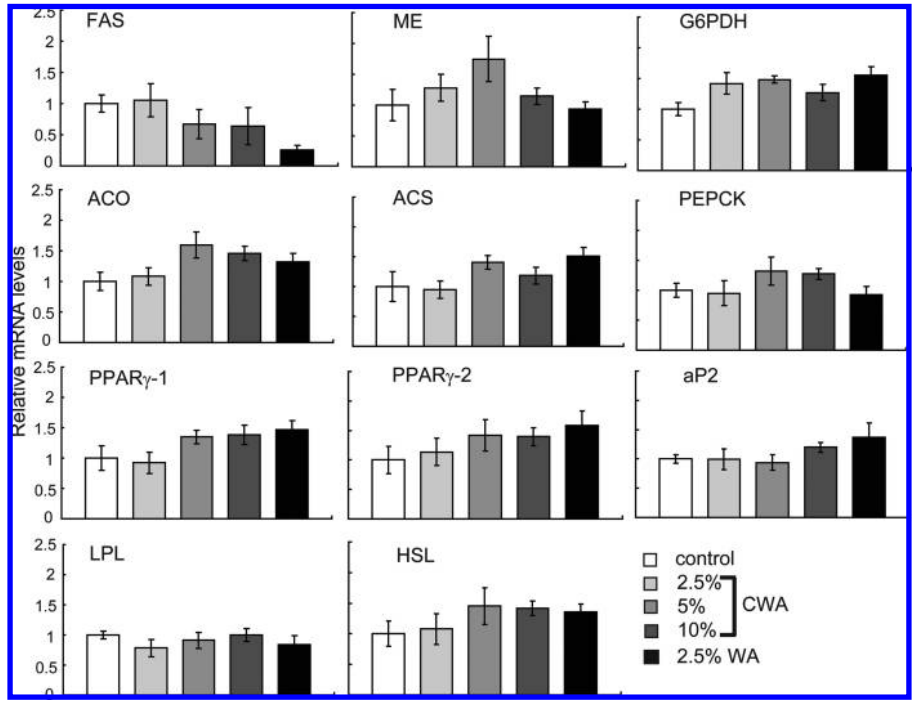

Figure 4. Effects of feeding rats diets supplemented with various concentrations of CWA or 2.5% WA on the expression of genes related to lipid metabolism and insulin sensitivity in epididymal adipose tissue. Each bar represents the mean ± SEM ($n = 5-7$).

showed that inhibition of postprandial hyperglycemia/hyperinsulinemia reduced lipogenic enzyme activities in the mesenteric adipose tissue as well as the triacylglycerol level (*11*). Thus, we examined the expression of these and other insulin sensitivity-related genes in the mesenteric and epididymal adipose tissue depots of rats fed diets containing WA or various concentrations of CWA. FAS, ME, and G6PDH are the major lipogenic enzymes involved in fatty acid synthesis (*14*), whereas ACO (*15*) and ACS (*16*) are key enzymes involved in β-oxidation. PEPCK in adipose tissues plays a role in re-esterification of fatty acids to produce triacylglycerol (*17*). PPARγ1 and PPARγ2 are major transcriptional factors required for adipocyte differentiation, and PPARγ2 was reported to exhibit higher transactivity and stronger induction of white adipocyte differentiation than PPARγ1 (*18*). aP2, a fatty acid-binding protein in white adipocytes, is a well-known marker for adipocyte

differentiation (*19*). LPL is an enzyme required for hydrolysis of triacylglycerol derived from VLDL/chylomicron to fatty acids and glycerol (*20*), whereas HSL is an enzyme involved in the hydrolysis of stored triacylglycerol in adipocytes to fatty acids and glycerol (*21*). Among the genes examined in this study, the mRNA levels of G6PDH, ACO, ACS, PEPCK, and LPL were significantly reduced in the mesenteric adipose tissue of rats fed the diet containing 2.5% WA. Furthermore, the G6PDH and LPL mRNA levels were significantly reduced in the mesenteric adipose tissue of rats fed the diet supplemented with 10% CWA. In addition, linear regression analyses showed that the FAS, G6PDH, ACS, and LPL gene expression levels in the mesenteric adipose tissue were reduced in dose-dependent manners according to the concentration of CWA in the diet (**Figure 3**). On the other hand, diets supplemented with CWA and WA did not cause any significant changes in the expression of genes related to lipogenesis and insulin sensitivity in the epididymal adipose tissue (**Figure 4**). These results suggest that supplementation with CWA as well as WA predominantly affected the expression levels of genes related to lipogenesis and insulin sensitivity in the mesenteric adipose tissue. Recent studies have suggested that white adipocyte differentiation is associated with PPARγ expression, especially PPARγ2 (*18*). It seems that PPARγ2 gene expression tends to be reduced by WA (**Figure 3**). In cell cultures, fibroblasts are rapidly differentiated within 1 week (*22*, *23*). In rodents, the adipose tissue matures during the transient suckling−weaning period from postnatal day 13 to day 27 (*24*). Thus, the decreased expression of genes related to lipogenesis and insulin sensitivity in rats fed diets supplemented with WA may be involved in the decreased white adipocyte differentiation observed after inhibiting postprandial hyperinsulinemia. Future studies need to investigate the effects of CWA/WA diets on gene expression of brown adipocyte differentiation markers in brown adipose tissue.

It is still unknown which factors are responsible for altering these expressional changes in rats fed the CWA/WA diet. It is reported that the action of insulin to regulate expression of genes related to lipogenesis and insulin sensitivity is transmitted by several kinases which induce phosphorylation, a post-translational modification, of proteins such as PI3 kinase under the control of insulin receptor substrates (*25*). Also, another kinase, AMP kinase, regulates gene expression under the guise of insulin sensitivity (*26*). These kinases enhance the expression of genes related to lipogenesis and insulin sensitivity by activating phosphorylation cascades. Future studies need to determine, using the phosphorylated forms of these kinases, whether these kinase cascades are involved in the repression of genes related to lipogenesis and insulin sensitivity by feeding rats the CWA/WA diets.

The reason mesenteric adipose tissue, but not epididymal adipose tissue, is sensitive to dietary manipulations of α-amylase inhibitors is unclear. It is likely that mesenteric adipose tissue may be especially vulnerable to insulin resistance compared with epididymal adipose tissue, but this hypothesis requires further examination in various animal models and human subjects.

It should be noted that CWA contains other compounds in addition to α-amylase inhibitor. Because the control diets are compensated by containing autoclaved−inactivated CWA, proteins that may reduce triacylglycerol levels and gene expression should be inactivated by the heat. Although other proteins with some activities may contribute to the reduction of triacylglycerol and mRNAs related to lipogenesis and insulin sensitivity, the α-amylase inhibitor activity in CWA should contribute most to these effects, because the α-amylase inhibitor activity in CWA is relatively high (**Figure 1**). Furthermore, we suggest that CWA has an advantage compared with WA because the procedure is simple. In addition, future studies need to investigate whether other proteins, except for the α-amylase inhibitor in CWA, have effects on reducing plasma triacylglycerol and expression of genes related to lipogenesis and insulin sensitivity.

In conclusion, the results of the present study suggest that dietary supplementation with CWA as well as WA exerts effects on the expression of genes related to lipogenesis and insulin sensitivity in mesenteric adipose tissue.

ABBREVIATIONS USED

ACO, acyl-CoA oxidase; ACS, acyl-CoA synthase; aP2, adipocyte fatty acid binding protein; CWA, crude preparation of wheat albumin; FAS, fatty acid synthase; G6PDH, glucose-6-phosphate dehydrogenase; HSL, hormone sensitive lipase; HPRT, hypoxanthine guanine phosphoribosyl transferase; LPL, lipoprotein lipase; ME, maleic enzyme; PEPCK, phosphoenolpyruvate carboxykinase; PPAR, peroxisome proliferator-activated receptor; WA, wheat albumin.

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