Note

Egg White Hydrolysate Improves Glucose Tolerance in Type-2 Diabetic NSY Mice

Masaru OCHIAI and Yoshiyuki AZUMA
School of Veterinary Medicine, Kitasato University, Higashi 23–35–1, Towada, Aomori 034–8628, Japan
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Summary We have previously reported that chicken egg white (EW) and low-allergenic EW hydrolysate (EWH) suppressed ectopic fat accumulation and improved serum glucose and insulin levels. In this study, the dietary effects of EW and EWH on glucose tolerance were investigated in different ways to clarify the effect of EW and EWH on intestinal glucose absorption. Type 2 diabetic Nagoya-Shibata-Yasuda mice were divided into four groups: a low-fat and low-sucrose casein-based diet group (NL); high-fat and high-sucrose (HFS) casein-based diet group (NH); HFS EW-based diet group (NE); and HFS EWH-based diet group (NEH). Mice were fed their respective diets for 8 wk. At the end of the 6th and 7th week, an oral glucose tolerance test (OGTT) and insulin tolerance test (ITT) were respectively conducted in experiment A. At the end of the 7th week, an intraperitoneal glucose tolerance test (ipGTT) was conducted in experiment B. In experiment A, the plasma glucose level was suppressed in the NE group during both OGTT and ITT, and suppressed in the NEH group during OGTT, but not during ITT. In experiment B, the plasma glucose level was similarly suppressed in the NEH group during ipGTT, but the suppressive effect was weakened compared to OGTT. Plasma insulin level was lower in the NE and NEH groups in both experiments. Fecal triacylglycerol excretion was increased in the NE and NEH groups in experiment A and liver triacylglycerol content was suppressed in the NE group in experiment B. These findings suggested that in addition to improving fat metabolism, EWH improves glucose tolerance via mechanisms related and unrelated to small intestinal function.

Key Words egg white hydrolysate, glucose tolerance, oral administration, intraperitoneal injection, Nagoya-Shibata-Yasuda mice

Chicken egg white (EW) makes up about 60% of the whole egg weight and contains proteins high in amino acid balance. EW has been reported to suppress food intake, dietary fat absorption, and fat accumulation in rats in our previous studies (1–3); therefore, EW can be considered for the prevention and treatment of obesity and type 2 diabetes mellitus (T2DM) in humans. However, egg-specific proteins in EW are well known to be major allergens in humans. Continuous consumption of EW or whole egg can induce allergic symptoms in humans; therefore, it is necessary to produce low-allergenic EW products. Recently, low-allergenic bioactive peptides derived from EW have been developed and purified for the prevention and improvement of several metabolic disorders such as hypertension and oxidative stress (4–6). However, few studies have reported the effects of low-allergenic EW products on glucose metabolism.

We previously developed EW enzymatic hydrolysate (EWH), which contains less allergenic proteins and has similar physicochemical properties to EW (3). We have also reported that EWH improves fasting serum glucose and insulin levels in diabetic Goto-Kakizaki (GK) rats (1) and Wistar rats (2). These findings suggest that in addition to improving fat metabolism, EWH can prevent the onset of glucose intolerance and T2DM. However, these effects of EWH and their mechanisms have not been elucidated.

In general, the ability of glucose tolerance has been investigated by the administration of glucose to animals orally or intraperitoneally (7). But if only intraperitoneal glucose administration is investigated, the possibility of intestinal functions related to glucose absorption and incretin secretion is excluded. On the other hand, when only oral glucose administration is investigated, the possibilities of both intestinal functions related to glucose absorption and peripheral glucose uptake are taken into consideration. Therefore, both oral and intraperitoneal glucose tolerance tests should be conducted to clarify the dietary effects of food materials on intestinal glucose absorption.

In this study, the effect of low-allergenic EWH on glucose tolerance was investigated from the aspect of small intestinal glucose absorption in spontaneously type-2 diabetic mice. Then, the effects of EWH on fat and carbohydrate metabolism were partially investigated under both fasting (experiment A) and non-fasting states (experiment B).

Methods and Materials

Test materials. Powdered EW and EWH (product

E-mail: mochiai@vmas.kitasato-u.ac.jp
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Name: Sunny Pro WP) were both donated by Taiyo Kagaku Co., Ltd. (Mie, Japan). EWH was previously produced by the protease treatment of EW and powdered. We have previously confirmed that EW-specific main allergens, particularly ovalbumin, ovotransferrin and lysozyme, are decreased to the level of about 20% in EWH compared to EW using SDS-PAGE and quantitative ELISA methods (3). As EW proteins were totally enzymatically hydrolyzed, specific peptides with unique amino acid sequences were not obtained in the EWH.

Casein was purchased from Fonterra Co. (Auckland, New Zealand). Nutritional components of casein, EW, and EWH were determined and the results were shown in Table 1.

Animals treatment and diets. This animal experiment was approved by the Committee of Kitasato University (Approval no. 15-143 and 16-140).

In experiment A, 32 diabetic Nagoya-Shibata-Yasu da (NSY) mice (male, 4-wk-old) were purchased from Hoshino Laboratory Animals, Inc. (Ibaraki, Japan), and 8 ICR mice (male, 4-wk-old) were purchased from Japan SLC, Inc. (Shizuoka, Japan). The NSY mouse established by selective breeding for glucose intolerance from the ICR mouse is generally known to be a spontaneous and inbred line model of T2DM (8). Mice were housed at 23 ± 2°C with lights on between 07:00 and 19:00. Free access to water and a chow diet (CE-2; CLEA Japan, Inc., Tokyo) was given to them for 1 wk of acclimation. NSY mice were divided into four dietary groups (8 mice per group): the low-fat and low-sucrose (LF) casein-based diet group (NL group); high-fat and high-sucrose (HFS) casein-based diet group (NH group); HFS EW-based diet group (NE group); and HFS EWH-based diet group (NEH group). ICR mice were fed the LF casein-based diet (IL group, n=8). Mice were fed their respective diets ad libitum for 8 wk. Dietary composition is shown in Table 1.

Table 1. Nutritional composition of the dietary proteins and diet composition.

| Nutritional composition | Casein | EW | EWH |
|--------------------------|--------|----|-----|
| **Protein (%)**          | 88.5   | 82.6| 80.3 |
| **Fat**                  | 0.0    | 0.0 | 0.2 |
| **Moisture**             | 10.7   | 6.8 | 8.3 |
| **Ash**                  | 0.8    | 5.9 | 5.9 |
| **Other**                | 0.0    | 4.7 | 5.3 |
| **Total**                | 100    | 100 | 100 |

| Diet composition | LF | HF |
|-------------------|----|----|
| **Ingredients (g/kg)** |       |     |
| Casein            | 200.0 | 250.0| —  |
| EW                | —   | —   | 268.0|
| EWH               | —   | —   | 275.5|
| dl-Methionine     | 3.0 | 3.0 | 3.0 |
| Cornstarch        | 529.5| 149.5| 131.8|
| Sucrose           | 100.0| 200.0| 200.0|
| Cellulose         | 50.0 | 50.0| 50.0 |
| Soybean oil       | 70.0 | 230.0| 230.0|
| Beef tallow       | —   | 70.0| 70.0 |
| AIN-93 vitamin mix| 10.0| 10.0| 10.0 |
| AIN-93G mineral mix| 35.0| 35.0| 35.0 |
| Choline chloride  | 2.5 | 2.5 | 2.5 |
| Butylhydroxytoluene | 0.014| 0.014| 0.014|
| **Total**         | 1,000| 1,000| 1,000|

| Energy composition (%) |       |     |
|------------------------|-------|-----|
| Carbohydrate           | 66    | 29  | 29  |
| Fat                    | 16    | 53  | 53  |
| Protein                | 18    | 18  | 18  |
| **Total**              | 100   | 100 | 100 |

| Energy (kcal/kg)² | 3.938 | 5.065 | 5.045 | 5.025 |

EW, egg white; EWH, egg white hydrolysate.

1 Other in each protein material was calculated as follows and is considered as carbohydrate: other (%) = total 100% — (protein + fat + moisture + ash).

2 Protein, fat, and carbohydrate provide 4, 9, and 4 kcal/g, respectively.
Body weight (BW) and energy intake in each group was monitored twice a week. At the end of the 6th week, an oral glucose tolerance test (OGTT) was conducted. After an overnight fasting, d-glucose (1 g/kg) was orally administered to each mouse. Before and 30, 60, 90, and 180 min after the d-glucose administration, blood (about 30 μL) was collected from tail vein, and centrifuged (6,200 × g, 4°C, 5 min) to obtain plasma. At the end of the 7th week, an intraperitoneal insulin tolerance test (ITT) was conducted. After the overnight fast, porcine insulin (0.33 IU/kg) was intraperitoneally administered to each mouse. Before and 30, 60, 90, and 180 min after the insulin administration, plasma was obtained as the same way as for OGTT. The plasma glucose level during OGTT and ITT was measured using a commercial kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Area under or over the curve (AUC or AOC) of the plasma glucose level was calculated by the trapezoidal rule. Feces collected during the final 3 d were freeze-dried and weighed. At the end of the 8th week, the mice were euthanized under isoflurane anesthesia after overnight fasting. Collected blood (about 1 mL) was centrifuged (6,200 × g, 4°C, 15 min) to obtain plasma. Liver, adipose tissues, and gastrocnemius muscles were quickly removed and weighed. Plasma and tissues were stored at −80°C until analyses.

In experiment B, 31 NSY mice (male, 4 wk-old) were purchased from Hoshino Laboratory Animals, Inc., and 10 ICR mice (male, 4 wk-old) were purchased from Japan SLC, Inc. Mice were treated and fed with the same diet as in experiment A (IL, n=10; NL, n=7; NH, NE, and NEH, n=8 in each group). At the 8th week, d-glucose (1 g/kg) was administered intraperitoneally (ipGTT) and plasma (about 30 μL) was taken in the same way as for OGTT for glucose analysis. At the end of experiment B, plasma and tissues were collected without fasting by the same methods as in experiment A.

**Biochemical analyses.** Plasma glucose level during OGTT, ITT, and ipGTT and final plasma levels of glucose, triacylglycerol (TG), total cholesterol (CHO) (Wako), glucagon-like peptide 1 (GLP-1), insulin (Shibayagi Co., Ltd., Gunma, Japan), and total adiponectin (Otsuka Pharmaceutical Co., Ltd., Tokyo) were measured using appropriate commercial kits. The homeostasis model assessment of insulin resistance (HOMA-IR) and insulin secretion (HOMA-β) was calculated as previously described (3). Total lipids in feces, liver, and gastrocnemius muscle were extracted as described by Folch et al. (9). The feces were powdered with a commercial food mill and the liver and gastrocnemius muscle were powdered with liquid nitrogen by a homogenizer, and then total lipids in the powdered samples were extracted with a mixture of chloroform and methanol (2:1, v/v). Total lipids in feces were measured by the volatilization of extracted lipids. TG and CHO contents in feces, liver, and gastrocnemius muscle were measured by the same method as for plasma analysis.

**Statistical analyses.** Statistical analysis on energy intake and fecal parameters could not be conducted because of the group housing of mice in each cage. Thus, data are expressed as means and actual data. The other data are expressed as mean±standard error (n=7–10). Statistical analysis of differences among the four NSY mice groups was performed using one-way analysis of variance and the Tukey-Kramer test. Statistical analysis of differences between IL and NL groups was performed using Student’s t-test. A difference with p<0.05 was statistically significant. All statistical analyses were performed using a commercially available statistical package (Excel Statistics 2015; SSRI, Tokyo, Japan).

**Results and Discussion**

T2DM is caused by a defect in insulin secretion and/or insulin action. NSY mice are late in onset of T2DM, and are moderately obese models with both impaired insulin response to glucose and insulin resistance (10). To the present, it has been clarified that three major quantitative trait loci mapped on chromosomes 11, 14, and 6 interacted to cause hyperglycemia and glucose intolerance in NSY mice (11, 12). Glucose intolerance was worsened in an age-dependent manner and a higher level of pancreatic insulin lasted to 36 wk of age (10, 13). Insulin response to both glucose and non-glucose stimuli in isolated pancreatic islets was markedly impaired (10, 14). These reports have presupposed that NSY mice have a defect in insulin secretion through an impaired ATP-sensitive K⁺-channel-dependent pathway. However, NSY mice at the age of 24 and 36 wk were found to be hyperinsulinemic as well as hyperglycemic (10, 13).

In the present study, the final plasma levels of glucose and insulin in both fasting and non-fasting states and HOMA indices at the age of 14 wk were significantly higher in the NL group than in the IL group. The plasma glucose levels during OGTT and ipGTT were worsened, but not significantly, in the NL group compared to the IL group. The plasma level of GLP-1, which is secreted by intestinal enteroendocrine L-cells and stimulates insulin secretion, was also significantly higher in the NL group than in the IL group. The present findings are consistent with a previous report that it is insulin-dependent peripheral glucose uptake that is impaired rather than the pancreatic functions in NSY mice (14). In addition, the peripheral insulin function and indices of gluconeogenesis and inflammation were furthermore worsened by a HFS diet in NSY mice (15, 16), which can be consistent with the present results. The plasma glucose level was significantly higher in the NH group than that in the IL group at 60, 90, and 180 min during OGTT and slightly higher during ITT (experiment A, Fig. 1), and significantly higher in the NH group than that in the NL group at 60 min during ipGTT (experiment B, Fig. 1). AUC or AOC of the glucose levels during OGTT, ipGTT, and ITT were also significantly different between the NH and NL groups (Fig. 1). The final fasting plasma glucose level was significantly higher in the NH group than that in the NL group (experiment A), and the insulin level under both fasting and non-fasting states was higher, but not significantly, in the NH group than that in the NL group (Table 2). The plasma total adiponectin level,
an index of insulin resistance secreted from adipose tissues, at a fasting state was also significantly lower in the NH group than in the NL group, although no differences existed in the adipose tissues weight between these two groups.

Glucose transporter (GLUT) 4 is a candidate gene for glucose uptake into muscles and located in the central region of chromosome 11. The nucleotide sequence of GLUT4 in NSY mice was reported to be identical to that in control mice (13), but influence on the impaired function of GLUT4 has not yet clarified. The defect in the function of GLUT4 is well known to induce insulin resistance. In non-obese and type 2 diabetic GK rats, which are similar to NSY mice in terms of impaired insulin secretion rather than insulin resistance, GLUT4 protein expression, glucose uptake, and glucose utilization in muscles were totally suppressed (17).

In experiment A, the EWH diet suppressed an increase in the plasma glucose level at 60 and 90 min and suppressed the AUC value at the level of 51% during OGTT compared to the HFS casein-based diet (Fig. 1). Comparable results were also caused by the EW diet (31%), but not significantly. Throughout ITT, the EW diet suppressed plasma glucose level and indicated a sig-
Table 2. Body weight gain, food intake, tissues weight, plasma components, and fat accumulation in Experiment A and B.

|                      | ICR mice | NSY mice |                | Low-fat diet | Low-fat diet | High-fat and high-sucrose diet |                |
|----------------------|----------|----------|----------------|--------------|--------------|--------------------------------|----------------|
|                      | IL       | NL       | NH             | NE           | NEH          |                                |                |
| **Experiment A**     |          |          |                |              |              |                                |                |
| Number of mice       | 8        | 8        | 8              | 8            | 8            |                                |                |
| Body weight gain and food intake |          |          |                |              |              |                                |                |
| Final body weight (g) | 49.8±1.7 | 44.7±1.3 | 48.6±0.6       | 46.2±1.0     | 46.0±1.2     |                                |                |
| Body weight gain (g) | 21.2±1.4 | 20.7±1.2 | 24.4±0.4       | 22.5±1.5     | 22.4±1.4     |                                |                |
| Energy intake (kcal/d) | 20.2 (19.7, 20.7) | 17.9 (17.5, 18.3) | 17.2 (16.9, 17.4) | 16.9 (16.6, 17.2) | 16.3 (16.2, 16.4) |                                |                |
| **Tissue weight (mg/g tissue)** |          |          |                |              |              |                                |                |
| Liver                | 38.9±1.2 | 36.6±2.1 | 39.8±1.9       | 37.6±1.0     | 36.2±1.7     |                                |                |
| Abdominal adipose tissue² | 71.9±6.2 | 84.5±4.2 | 79.3±5.0       | 82.9±2.1     | 93.5±2.9     |                                |                |
| Gastrocnemius muscle | 7.6±0.4  | 6.6±0.2  | 6.5±0.2        | 6.7±0.1      | 7.3±0.7      |                                |                |
| **Serum biochemical components** |          |          |                |              |              |                                |                |
| Glucose (mmol/L)     | 101±6.3  | 97±1.4   | 103±7.2        | 105±6.6      | 109±5.6      |                                |                |
| Glucagon (pg/mL)     | 100±5.2  | 80±4.3   | 90±5.7         | 95±4.9       | 100±5.6      |                                |                |
| **Experiment B**     |          |          |                |              |              |                                |                |
| Number of mice       | 10       | 7        | 8              | 8            | 8            |                                |                |
| Body weight gain and food intake |          |          |                |              |              |                                |                |
| Final body weight (g) | 51.5±1.5 | 43.9±0.5 | 48.9±1.3       | 45.7±1.7     | 48.0±1.2     |                                |                |
| Body weight gain (g) | 17.7±1.4 | 15.5±0.6 | 19.6±1.2       | 17.2±1.6     | 18.8±1.4     |                                |                |
| Energy intake (kcal/d) | 20.8 (20.6, 20.9) | 15.3 (14.7, 15.9) | 16.0 (15.6, 16.4) | 15.3 (15.2, 15.5) | 14.6 (14.6, 15.1) |                                |                |
| **Tissue weight (mg/g tissue)** |          |          |                |              |              |                                |                |
| Liver                | 44.4±1.4* | 37.5±2.7 | 41.1±2.2       | 40.8±2.1     | 39.5±1.4     |                                |                |
| Abdominal adipose tissue² | 64.2±5.0* | 85.3±3.2 | 86.0±4.1       | 84.5±5.0     | 94.5±2.4     |                                |                |
| Gastrocnemius muscle | 7.6±0.4  | 6.7±0.1  | 6.2±0.2        | 6.9±0.3      | 6.5±0.1      |                                |                |
| **Serum biochemical components** |          |          |                |              |              |                                |                |
| Glucose (mmol/L)     | 10.6±0.5* | 13.1±0.5 | 14.3±2.3       | 12.4±0.4     | 12.7±0.5     |                                |                |
| Glucagon (pg/mL)     | 58±13    | 248±144ab | 1,340±532a     | 82±14b       | 235±94b      |                                |                |
| **Body weight gain and food intake** |          |          |                |              |              |                                |                |
| Final body weight (g) | 34.6±4.6 | 46.5±7.0b | 89.3±14.7b     | 49.0±4.2b    | 63.8±7.4b    |                                |                |
| Body weight gain (g) | 7.4±0.3  | 7.2±0.3a | 7.5±0.5b       | 6.0±0.2b     | 6.6±0.3b     |                                |                |
| **Data**             |          |          |                |              |              |                                |                |

Values are mean±standard error (n=8 mice, experiment A; n=7–10, experiment B). Statistically significant differences were evaluated by one-way ANOVA and the Tukey-Kramer test among the four NSY mice groups. Means with different letters are significantly different at p<0.05. Significant differences between the IL and NL groups were evaluated by Student’s t-test (* p<0.05).

¹ Mean value was given on the left and actual value of each cage was given in parenthesis; statistical analysis could not carried out because of group housing of mice in each cage.

² Sum of perirenal, epididymal, and mesenteric adipose tissue weight.

³ Values of homeostasis model assessment (HOMA) of IR (HOMA-IR) and insulin secretion (HOMA-B) were calculated using the following formulae: HOMA-IR=fasting insulin (μIU/mL)×fasting glucose (mmol/L)/22.5 and HOMA-B=20×fasting insulin (μIU/mL)/fasting glucose (mmol/L)×3.5.

GLP-1, glucagon like peptide-1; TG, triacylglycerol; CHO, cholesterol.
significant increase in the AOC at the level of 46% compared to the HFS casein-based diet group. The EWH diet did not change plasma glucose levels throughout ITT. ITT is an evaluating method for systemic insulin resistance, whereas OGGT and ipGTT are for glucose tolerance and insulin secretion ability. These results have indicated that the EWH diet improves glucose tolerance and insulin secretion, resulting in a decrease in plasma glucose levels only when glucose is given. In experiment B, the plasma glucose level was significantly lower in the NE group at 90 and 180 min, and slightly lower in the NEH group than that in the NH group during ipGTT. The AUC value during ipGTT was significantly or slightly ($p=0.06$) lower in the NE and NEH groups (36% and 21%, respectively) than that in the NH group. The plasma insulin level under both fasting and non-fasting states and HOMA-IR index were also significantly or slightly lower in the NE and NEH groups than that in the NH group, which is consistent with our previous finding (1). However, no significant differences were observed in the plasma glucose levels during OGTT or ipGTT, or in plasma levels of glucose or insulin and HOMA indices under both fasting and non-fasting states between the NE and NEH groups.

These results during ipGTT are comparable to those during OGTT, but the EWH diet-induced suppressive effect on plasma glucose levels was weakened in OGTT. There is a difference of meaning between ipGTT and OGTT: administration of glucose via an intraperitoneal injection means that there is no incretin response that potentiates a glucose-mediated insulin response. From the present results, the EWH-induced improvement of small intestinal functions such as incretin secretion has been considered. For the improvement of glucose metabolism, incretin secretion stimulants and dipeptidyl peptidase-4 inhibitors from food proteins such as rice, wheat, and zein have been investigated (18–20). EWH also stimulates the release of GLP-1 in STC-1 cells (21). However, the plasma total GLP-1 level in both fasting and non-fasting states and the fasting plasma adiponectin level were not unexpectedly changed by the EW and EWH diets (Table 2). In our previous study (1), the plasma activity of dipeptidylpeptidase-4, an enzyme to inactivate GLP-1, was not changed by EWH in GK rats. These results indicate that the suppression of glucose levels in the NEH group during OGTT and ipGTT was not caused by glucose-mediated secretion of incretin and insulin and adiponectin pathways, but caused by both small intestine-dependent and -independent mechanisms. It should be clarified whether EWH suppresses the intestinal glucose absorption and/or increases peripheral glucose uptake in a future study.

One of the apparent differences between EW and EWH was the average molecular weight of each protein. In our recent report (3), ovalbumin, lysozyme, and ovotransferrin, which are EW-derived allergenic proteins, were enzymatically hydrolyzed to lower allergenic hydrolysates. However, EWH maintained the suppressive effect of EW on lipid absorption in rats fed the HFS diet (3). Moreover, the dispersibility of EWH in water-oil emulsion was notably higher compared to casein and EW, which indicates that EWH suppresses enzymatic activities of fat and carbohydrate digestion by binding to enzymes in small intestine. However, it has not yet reported that EW and EWH suppress the digestion of dietary fat and carbohydrate in an in vivo study.

It has been thought possible that food proteins and their hydrolysates can stimulate peripheral glucose uptake and decrease the blood glucose level. Soy β-conglycinin, a constituent of soy proteins, has been reported to increase glucose uptake in muscles by activating the translocation of GLUT4 and AMP-activated protein kinase expression in GK rats (22). Whey protein hydrolysate has also been reported to increase the translocation of GLUT4 in muscles, which can induce glycogen accumulation in muscles (23). However, EW-derived specific peptides to stimulate glucose uptake in tissues have not yet been found. To clarify the mechanism for improvement of glucose tolerance and plasma insulin level by the EWH diet, the effects of EWH on peripheral glucose uptake should be investigated.

Energy intake was comparable among the HF diet groups, resulting in no significant differences in abdominal fat weight or body weight gain (Table 2). However, liver TG accumulation was accelerated by the HFS diet, which was consistent with a previous report (24), suggested that a locus for fatty liver mapped on chromosome 6 contributed to liver steatosis independent of body weight gain or adipose tissue accumulation in NSY mice. Strong correlations between fatty liver and glucose intolerance were revealed in NSY mice (24). The mRNA expression of peroxisome proliferator-activated receptor γ (PPARγ), a candidate regulator of adipocyte differentiation and lipid storage, in the liver was up-regulated with a high-sucrose diet (15), and a part of the sequence of PPARγ was different between NSY and control mice, although the influence of the difference has not yet elucidated (24). Liver TG content under both fasting and non-fasting states was slightly ($p=0.06$, experiment A) or significantly (experiment B) increased by the HFS diet. The EW and EWH diets did not suppress the liver TG accumulation in a fasting state, but the EW and EWH diets significantly and slightly suppressed liver TG content, respectively, in a non-fasting state (Table 2). Fasting largely affects lipid metabolism, particularly TG synthesis, in mice (25). TG content and enzymatic activity and mRNA expression related to lipogenesis in the liver were decreased in a fasting-time-dependent manner. Therefore, liver TG content in the NH group was supposed to be lower in the case of overnight fasting compared to that of non-fasting. EW acted as a pancreas lipase inhibitor and suppressed intestinal TG absorption, resulting in a decrease in liver TG content (26), which was comparable in the present experiment B. Our report previously also showed that the enzymatic activities related to lipogenesis in the liver were suppressed together with lowered liver TG content in rats fed the HFS diet (2). Therefore, EW can be a protein to suppress TG accumulation in the body. One of reasons why the suppressive effect of EW on liver TG content
was weakened in the case of EWH is considered to be that lipase inhibitors in EW such as ovalbumin were previously hydrolyzed (27).

The EW diet also significantly lowered liver CHO content, and the EWH diet suppressed liver CHO content equal to or lower than the level of the LF diet group. Fecal excretion of TG and CHO was numerically higher in the NE and NEH groups than in the NH groups although the fecal weights and fecal total lipids content were equal (Table 2). From the results of lipids analyses, EW clearly suppressed CHO accumulation, which is supposed to be caused by lower lipid absorption and fat synthesis (1–3, 26). EWH slightly suppressed the CHO accumulation, but the effect was weakened compared to EW. In our previous studies (1–3), fecal TG excretion was higher in the EW and EWH diets. As the EW-induced mechanism for the suppressing intestinal fat absorption, inhibiting micellar formation and lipid transfer were suggested (26). We have suggested that the higher dispersibility of EWH as well as EW in the digestive tract could suppress the pancreatic lipase activity, resulting in higher fecal fat excretion (3). Ovalbumin, an abundant protein in EW, was responsible for the lowering of TG and CHO because ovalbumin was resistant to pepsin digestion in the gut and passed through the intestine and had a lipase inhibitory activity (26, 27). However, ovalbumin as well as ovotransferrin and lysozyme in the EWH used in the present study was previously hydrolyzed enzymatically according to our previous report (3). The higher excretion of fecal TG and CHO in the EWH diet group can be caused by other proteins and hydrolyzed peptides in EW via inhibition of lipid digestion, intestinal absorption, and/or chylomicron secretion, but their candidate proteins or peptides have not yet been clarified.

To conclude, in addition to suppressive effects on lipids accumulation in the liver, low-allergenic EWH is expected to improve glucose tolerance in type-2 diabetic NSY mice via both small intestine-related and unrelated mechanisms. The EWH-induced improvement of glucose tolerance was higher in OGTT than in ipGTT. Effects of EWH on intestinal glucose absorption and other intestinal functions as well as peripheral glucose uptake should be clarified.

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