Egg White Hydrolysate Can Be a Low-Allergenic Food Material to Suppress Ectopic Fat Accumulation in Rats Fed an Equicaloric Diet

Masaru Ochiai1,2, Kohei Misaki2, Toshiki Takeuchi2, Ryoyo Narumi1, Yoshiyuki Azuma1 and Tatsuhiro Matsuo2

1 School of Veterinary Medicine, Kitasato University, Higashi 23–35–1, Towada, Aomori 034–8628, Japan
2 Faculty of Agriculture, Kagawa University, Ikenobe 2393, Miki, Kita, Kagawa, 761–0795, Japan
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Summary Egg white (EW) is known as a nutritional protein but can induce allergic reactions in humans. We investigated the dietary effects of EW and its hydrolysate (EWH), which contains less allergen, on body fat accumulation in Wistar rats fed an equicaloric high-fat and high-sucrose diet for 8 wk (Exp A). The pair-feeding of EW and equicaloric-feeding of EWH increased fecal fat excretion and suppressed lipid accumulation in the liver and muscles but not in the abdominal adipose tissues, carcass, or total body. Dietary EWH also suppressed the serum glucose level and alkaline phosphatase activity. Further, we showed a higher dispersibility of EW and EWH in physicochemical assay (Exp B). Next, we investigated the suppressive effects of a single administration of EW and EWH on lipid-induced hypertriglyceridemia and small intestinal meal transit in ddY mice (Exp C). However, a single administration of EW or EWH did not suppress the lipid-induced hypertriglyceridemia nor did it delay the rate of small intestinal transit. These findings indicated that dietary EW and EWH reduce hepatic and muscular (ectopic) fat accumulation mainly by suppressing fat absorption and supplying fat to the liver and muscles. Therefore, the low-allergenic EWH can be effective for the prevention of high-fat-diet-induced obesity.

Key Words egg white, hydrolysate, allergen, fat, equicaloric feeding

Obesity induced by excess fat intake and total body fat accumulation can lead to the development of insulin resistance (IR). In particular, ectopic fat accumulation, i.e., the fat accumulation in muscles, liver, and other nonadipose tissues, is an important risk factor of IR and type-2 diabetes mellitus (T2DM).

Dietary proteins have been shown to improve diet-induced obesity and IR (1). Egg white (EW) contains a high amount of protein with a high amino acid balance and is used as a physicochemical functional and nutritional food ingredient, but the allergenicity of EW proteins in humans is well known. Food allergy occurs more frequently in children than in adults, and its prevalence varies among countries. Ovomucoid, ovalbumin, ovotransferrin, and lysozyme have been identified as major EW allergens (2). In particular, ovomucoid shows heat- and acid-stable characteristics and possesses strong allergenicity. Ovalbumin is the most abundant protein in EW, but it is heat-labile and undergoes conformational changes to become more stable, thereby making it less allergenic. As ovalbumin, ovotransferrin, and lysozyme are also heat-labile proteins, the risk of a clinical allergic reaction to cooked egg or EW is thought to be relatively low (2). However, it is necessary to devise a method to reduce egg allergenic proteins in food processing as some people often eat raw or undercooked eggs in their diet.

EW hydrolysate (EWH) obtained by protease treatment is expected to be soluble in water, to be more quickly digested and absorbed in the small intestine, and to have some higher nutritional values (3). However, few reports have investigated the nutritional effects of EWH. In our previous study, dietary EWH decreased triacylglycerol (TAG) accumulation in muscles and liver in spontaneous diabetic Goto-Kakizaki rats (4). We also showed suppressive effects of EW and EWH on fat accumulation not only in the liver and muscles but also in intra-abdominal adipose tissues and carcasses of normal rats fed a high-fat and high-sucrose diet (HFSID) ad libitum (5). As a potential mechanism, it has been considered that dietary EW and EWH (particularly EW) can suppress food intake, the apparent rate of intestinal fat absorption, and some enzymatic activities in the liver and muscles, which are closely related to fat synthesis. Therefore, EW and EWH may be useful dietary protein materials for improving obesity, IR, and T2DM. However, the regulatory mechanism was not completely elucidated because the food intake was not equally controlled throughout the test period between the rats fed a casein diet and rats fed EW and/or EWH diets (5).

In the present Exp A, the suppressive effects of dietary EW and low-allergenic EWH on fat accumulation in muscles, liver, carcass, and total body were evaluated in rats fed an equicaloric diet. In Exp B, the biophysical
and physicochemical properties of EW and EWH were investigated in an in vitro study. In Exp C, the effects of a single administration of EW and EWH on lipid-induced hypertriglyceridemia and small-intestinal transit were investigated in lipid-induced hypertriglyceridemia ddY mice.

**MATERIALS AND METHODS**

Test materials. Casein was purchased from Fonterra Co. (Auckland, New Zealand). Powdered EW and EWH (Sunny pro WP) were both donated by Taiyo Kagaku Co. Ltd. (Mie, Japan), and EWH was obtained from protease treatment of EW. The molecular weight patterns of each dietary protein material were determined using sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The egg-derived allergenic protein content in the EW and EWH was quantified using a commercial kit (Fast Kit Elisa Ver. 3 Egg; NH Food Ltd., Ibaraki, Japan). EW-specific allergenic proteins such as ovalbumin (44.5 kDa), ovotransferrin (77.7 kDa), lysozyme (14.3 kDa), and ovomucoid (~28.0 kDa) (2), more or less disappeared and degraded to smaller nonallergenic proteins in the EWH (Fig. 1). Based on the quantitative analysis of egg allergenic proteins, the EW and EWH contain egg-specific allergenic proteins of 15.9 and 3.1 mg/g, respectively. The protein content of the casein, EW, and EWH was determined using the Kjeldahl method, with an N-to-protein conversion factor of 6.25. The total lipid content in the casein, EW, and EWH was determined using the Soxhlet method using a Soxtec™ 2055 Fat Extraction System (Foss Japan, Tokyo, Japan). The amount of moisture in the casein, EW, and EWH was determined as the loss in weight after drying at 105˚C for 24 h. The ash content in the casein, EW, and EWH was determined using the direct ignition method (550˚C for 24 h). The residues were estimated as carbohydrate content and classified as other. Nutritional components in each diet are shown in Table 1.

**Exp A: Suppressive effects of EW and EWH on fat accumulation in rats fed an equicaloric diet**

**Animals and Experimental diets.** All procedures involving the rats were approved by the Experimental Animal Care Committee of Kagawa University (Approval No.
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Eighteen male 3-wk-old Wistar rats were purchased from Japan SLC, Inc. (Shizuoka, Japan). The rats were individually housed at 22 ± 2˚C with lights on between 8:00 and 20:00 and were given free access to water and a commercial diet (type MF; Oriental Yeast Co., Ltd., Tokyo) for 13 d of acclimation. The rats were placed into three dietary protein groups: casein diet (C group, n = 6), EW diet (EW group, n = 7), and EWH diet (EWH group, n = 7). The rats were fed a HFSD (Table 1) for 8 wk. The rats in the EW groups were pair-fed with those in the C group throughout the test period. The rats of the EWH group were fed ad libitum throughout the test period because food intake of the rats fed a EWH diet was not significantly different from that fed a casein diet in our previous studies (4, 5). The body weight (BW) and food intake of each rat were monitored daily. Food efficiency was calculated by dividing BW gain by total food intake.

Collections of feces, blood, and tissues. During the final 3 d of the treatment period, feces were collected, freeze-dried, and weighed. At the end of the test period, all the rats were euthanized by decapitation after a 12 h fast. Blood was collected and centrifuged (6,200 × g, 15 min) to obtain the serum. The liver, adipose tissues (perirenal, epididymal, and mesenteric), and muscles (soleus, plantaris, gastrocnemius, extensor digitorum longus, and tibialis anterior) were quickly removed and weighed. The serum and tissues were then stored at −80˚C until analyses. The internal organs, legs, and tails were removed, and the remaining parts were defined as carcass and stored at −20˚C until analyses.

Biochemical analyses.

Serum component: The serum levels of glucose, insulin, alkaline phosphatase (ALP), leptin, nonesterified FAs (NEFAs), TAG, total cholesterol (CHO), and high-density lipoprotein (HDL)-CHO were measured using commercial kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The serum levels of insulin and leptin were measured using commercial enzyme-linked immunosorbent assay kits (Shibayagi Co. Ltd., Gunma, Japan). The values of homeostasis model assessment (HOMA) of IR (HOMA-IR) and insulin secretion (HOMA-β) were calculated using the following formulae (6): HOMA-IR = fasting insulin (μIU/mL)×fasting glucose (mmol/L)/22.5 and HOMA-β = 20×fasting insulin (μIU/mL)/(fasting glucose (mmol/L) − 3.5).

Lipid component: Total lipids in the feces, liver, and soleus muscle were extracted by Folch’s method (7). Total lipids in the feces were measured by volatilization of the extracted lipids. The TAG content in the total lipids from the feces, liver, and soleus muscle and CHO content in the feces and liver were measured using commercial kits (Triglyceride E and Cholesterol E, respectively; Wako Pure Chemical Industries, Ltd.). Total bile acids (TBA) in the feces were extracted using ethyl alcohol (65˚C for 1 h) and then measured using a kit (TBA: Wako Pure Chemical Industries, Ltd.). The apparent rate of fat absorption was calculated as follows: apparent absorption rate of the fat (%) = 100−[(fecal fat content/dietary fat intake)×100]. FA composition (C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, and C20:4) of the total lipids in the liver, soleus muscle, and perirenal adipose tissue was determined using gas chromatography (Shimadzu Co., Kyoto, Japan) in a 25-m capillary column (ULBON HR-20 M; Shimadzu Co.) as previously described (4). Indices of stearoyl-CoA desaturase (SCD) activity (ratios of C16:1/C16:0 and C18:1/C18:0) were calculated. Carcass fat was measured as described by Mickelsen and Anderson (8), and total body fat was calculated using a formula reported by De Bont et al. (9). Carcass moisture was measured after drying at 105˚C for 1 h. Glucose-6-phosphate dehydrogenase (G6PDH) activities of the liver and muscles (gastrocnemius and soleus) and fatty acid synthase (FAS) activity of the liver were measured according to methods previously described (10, 11).

Exp B: Biophysical and physicochemical properties of EW and EWH

Viscosities of EW and EWH. The viscosity of the solution containing casein, EW, and EWH was measured in triplicate using a Brookfield viscometer (TV-20; Toki Sangyo Co. Ltd., Tokyo, Japan) under the following conditions: 0.5–50 rpm and 25˚C for 20 s. Each solution was prepared to approach the conditions of lipid digestion and absorption in the digestive tract, with each protein sample (1 g) blended with 20 mL of 0.5% (w/v) carboxymethylcellulose (CMC), 10 mL of soybean oil (Showa Sangyo Co., Ltd., Tokyo, Japan), and 6.6 mM sodium cholate for 15 min. Shear velocity was calculated by multiplying each number of revolutions by the numeric constant 3.83. A blank control solution was prepared without any protein samples.

Micellar solubility of cholesterol and TAG in vitro. The micellar solubility of cholesterol (CHO) was measured using a modified enzymatic absorption spectrophotometry method (12). In short, 45 mM sodium taurocholate, 3.5 mM soya-derived lecithin, 1.2 mM CHO, 10 mM oleic acid, 3.6 mM monoolein, and 0.45 mM triolein in 15 mM sodium phosphate buffer (pH 6.8) was prepared by ultrasonication for 20 min using a Digital Sonifier (BRANSON 250D-advanced; Central Science Trade Co. Ltd., Tokyo, Japan). The micelles were maintained at 37˚C for at least 24 h for stabilization of the micelles. Each sample (casein, EW, and EWH) was suspended with 0.5% CMC and added at a sample concentration of 40 mg/mL to the micelles. The samples were incubated at 37˚C for 4 h and centrifuged (10,000 × g, 60 min) to obtain the upper phase. Levels of CHO and TAG in the upper phase were enzymatically measured using a commercial kit (Wako Pure Chemical Industries, Ltd.).

Exp C: Effect of a single administration of EW and EWH on lipid-induced hypertriglyceridemia and small-intestinal transit in mice

Animals. All procedures involving the rats were approved by the Experimental Animal Care Committee of Kagawa University (Approval No. 2014-31). Forty-three 7-wk-old male ddY mice were purchased from Japan SLC, Inc. The ddY mice have been reported to be an appropriate animal model for investigation of lipid-induced hypertriglyceridemia (13). The mice were treated for 3–4 d of acclimation under the same condi-
At 0, 90, 180, 270, and 360 min after the oil administered to all the mice. Blood was collected oil (Nacalai Tesque, Inc., Kyoto, Japan) (5 mL/kg) was minutes after the samples were administered, soybean the C group were administered 0.5% CMC only. Thirty were fasted for 12 h with free access to water. The mice concentration. The blood was centrifuged (6,200 g) for 5 min) to obtain plasma. Plasma TAG levels were measured using a commercial kit (Triglyceride-E test), and the area under the curve was calculated. After the oral lipids loading test, the mice were re-fed with the MF diet for 1 wk until the small-intestinal transit test.

Small-intestinal transit test. The small-intestinal transit test was carried out according to a previously reported method (14). Half of the mice in each group were fasted for 12 h with free access to water. The mice

| Table 2. Body weight gain, food intake, tissue weights, and serum components. |
|---------------------------------|----------------|----------------|
|                                | C              | EW             |
| Body weight gain and food intake |                |                |
| Body weight gain (g)            | 184.4±8.3      | 184.6±4.4      | 180.8±6.3      |
| Food intake (g/d)               | 11.5±0.3       | 11.5±0.3       | 11.0±0.1       |
| Food efficiency (g/g)           | 0.286±0.005    | 0.284±0.007    | 0.294±0.007    |
| Tissue weights (mg/g body weight)|                |                |
| Liver                           | 29.6±0.4       | 28.0±0.6*      | 28.0±0.4*      |
| Intra-abdominal adipose tissue   | 73.1±3.0       | 66.3±5.7       | 74.6±5.2       |
| Total hindlimb skeletal muscles  | 16.0±0.3       | 16.6±0.3       | 16.0±0.3       |
| Serum biochemical components    |                |                |
| Glucose (mg/100 mL)             | 147.9±2.6      | 129.1±3.7**    | 135.4±5.6*     |
| Insulin (μg/L)                  | 2.5±0.4        | 2.3±0.3        | 3.1±0.5        |
| HOMA-IR                         | 23.7±3.3       | 19.4±2.6       | 26.8±4.3       |
| HOMA-β                          | 281.3±44.4     | 325.8±26.6     | 396.5±59.9     |
| ALP (IU/L)                      | 267.2±12.0     | 195.8±12.3***  | 209.0±6.3**    |
| Leptin (pg/mL)                  | 380.2±94.2     | 133.5±22.3(*)  | 279.3±114.1    |
| TAG (mg/100 mL)                 | 110.4±10.5     | 95.1±10.2      | 100.1±21.5     |
| NEFA (mEq/L)                    | 0.79±0.06      | 0.86±0.06      | 0.91±0.06      |
| Total-CHO (mg/100 mL)           | 151.7±5.2      | 139.5±7.1      | 135.5±5.0*     |
| HDL-CHO (mg/100 mL)             | 86.9±3.9       | 76.0±3.9       | 66.6±1.6       |
| Non-HDL-CHO (mg/100 mL)         | 66.8±2.6       | 61.6±3.7       | 68.9±4.4       |

Values are mean±SE of 6 rats. Statistically significant differences were evaluated by one-way ANOVA and the Fisher-PLSD test. (*), **, *** p<0.1, 0.05, 0.01, 0.001, respectively (vs. C group).

1. Food efficiency and energy requirement were calculated by using a following formula: Food efficiency (g/g)=Body weight gain (g)/Food intake throughout the test period (g).
2. Sum of perirenal, epidymal, and mesenteric adipose tissue weight.
3. Sum of soleus, plantaris, gastrocnemius, tibialis anterior, and extensor digitorum longus muscles.
4. Values of homeostasis model assessment (HOMA) of IR (HOMA-IR) and insulin secretion (HOMA-β) were calculated using following formulae: HOMA-IR=fasting insulin (μIU/mL)×fasting glucose (mmol/L)/22.5 and HOMA-β=20×fasting insulin (μIU/mL)/(fasting glucose (mmol/L))×3.5.
5. Activity unit was shown as p-nitrophenol (mmol/L) produced from p-nitrophenylphosphate by enzymatic reaction for 1 min at 37°C.

ALP, alkaline phospatase; TAG, triacylglycerol; NEFA, non-esterified fatty acids; CHO, cholesterol; HDL, high-density-lipoprotein.

oral lipids loading test. After a 12 h fast, the mice were placed into three groups: control (n=15), EW (n=15), and EWH (n=13). The mice in the EW and EWH groups were administered the EW and EWH (500 mg/kg) suspended in 0.5% CMC, respectively. The mice in the C group were administered 0.5% CMC only. Thirty minutes after the samples were administered, soybean oil (Nacalai Tesque, Inc., Kyoto, Japan) (5 mL/kg) was orally administered to all the mice. Blood was collected from the tail vein before the sample administration and at 0, 90, 180, 270, and 360 min after the oil administration. The blood was centrifuged (6,200 g for 5 min) to obtain plasma. Plasma TAG levels were measured using a commercial kit (Triglyceride-E test), and the area under the curve was calculated. After the oral lipids loading test, the mice were re-fed with the MF diet for 1 wk until the small-intestinal transit test.

Small-intestinal transit test. The small-intestinal transit test was carried out according to a previously reported method (14). Half of the mice in each group were fasted for 12 h with free access to water. The mice in the EW and EWH groups were orally administered the EW and EWH (500 mg/kg), respectively, suspended in 0.5% CMC. The mice in the C group were orally administered 0.5% CMC only. Thirty minutes after the sample administrations, a test meal solution [0.5 mL of 5% (w/v) Evans blue suspended in 0.5% CMC] was orally administered. The test meal solution was prepared by adding 0.5% CMC to the AIN-93G diet (5%, w/v). Five minutes after the test meal solution was given, the mice were euthanized by decapitation. The small intestine from the pylorus to the ileocecal junction was removed, and the total length of the small intestine and the point to which the test meal solution had reached were measured. Small-intestinal transit was calculated as the ratio of the distance reached by the test meal solution to the total length of the small intestine and expressed as a percentage.

Statistical analyses. Each value is expressed as the mean±standard error [Exp A: n=6 rats; Exp B: n=3; Exp C (lipids-loading test): n=13–15 mice; Exp C (small-intestinal transit test): n=6–9 mice per group]. Statistical analysis of the differences among the three groups
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and EWH groups were observed, indicating that differences in BW gain or food intake between the EW and the same amounts of food (Table 2). No significant differences in intra-abdominal adipose tissue weight, and muscle body fat accumulation in rats. BW gain, food efficiency, and EW or EWH groups when all groups were provided weight were not significantly different between the C treatment from EW would be less allergenic and suppress the differences in molecular weight size did not influence the food intake or BW gain.

The serum leptin level was remarkably lower in the EW group than in the C group (65% suppression, \( p<0.05 \), Table 2), but not significantly different because of the huge variability in the data. Leptin secreted from abdominal adipose tissues is closely regulated by the adipose tissue weight (15) and plays an important role in energy homeostasis by regulating energy intake and energy expenditure. Obese animals complicated with T2DM exhibit high serum leptin levels and leptin resistance (16). However, the serum leptin level was not related to the intra-abdominal adipose tissue weight or energy intake in this study. Furthermore, the serum lipid levels and fat accumulation in the carcass and total body were not significantly different among the groups. Lower leptin levels in the serum of rats equicaloric-fed the EW diet may be associated with the suppression of appetite observed in our previous study (5).

Our previous study (5) indicated that the dietary EW-induced decreases in the food efficiency, body fat accumulation, and serum leptin level were mainly due to enhancing total lipid excretion in the feces and decreasing intestinal fat absorption in rats ad libitum fed a diet. The equicaloric feeding of the EW and EWH in the present study has also had an impact on dietary lipids absorption and fat accumulation. As well as the fecal TAG and cholesterol content, fecal TBA excretion was increased by dietary intake of the EWH compared with the casein diet. However, no significant differences in the fecal lipid excretion or apparent rate of fat absorption were observed between the EW and EWH groups. These results have indicated that EW suppressed intestinal fat absorption and specific EW-derived proteins did not show the differences in fecal lipid excretion (Table 2). To explain the suppression of apparent intestinal fat absorption, Matsuoka et al. (12, 17) suggested that EW decreased CHO absorption by inhibiting micellar formation and lipid transfer in the small intestine based on biochemical and nutritional properties. In a biophysical investigation, EW was shown to increase the water-holding capacity, volume in water, and viscosity compared with casein (12). However, in the previous report (12), lipid solubility and lipid transport in the digestive and absorption process were mainly focused on, and excreted fecal composition was not investigated. In this Exp A, fecal lipid excretion was enhanced by the equicaloric intake of the EW and EWH without increasing the excretion of the feces.

As a possible mechanism of suppressing intestinal lipid absorption and serum lipid levels by dietary EW, Wang et al. (18) and Hosomi et al. (19) respectively suggested that dietary proteins strongly bound to bile acids, and inhibited the micellar solubility of CHO compared with casein and/or its hydrolysate. Kobayashi et al. (20) also suggested that the solubility of CHO in the bile acid micellar juice is decreased by the binding of dietary polyphenol to phosphatidylcholine, but not CHO, in vitro. In this study, the effects of EW or EWH on viscosity and micellar solubility of lipids were investigated in vitro. The viscosity in emulsion was dramatically lower in the EW and EWH groups than in the C group and was also lower in the EWH than in the EW group in the wide range of shear velocity (Fig. 2A). The lower viscosity of the EW or EWH can indicate a higher dispersive activity in the digestive tract. Which can be similarly observed in the case of a lipase inhibitor (e.g. water-soluble fiber) (21). It is possible that lower viscosity in the digestive tract leads to higher inhibition of lipid digestion and

was performed using one-way analysis of variance and Fisher’s protected least significant difference test in Exps A and C. A difference with \( p<0.05 \) was considered to be statistically significant. All statistical analyses were performed using a commercially available statistical package (Excel Statistics 2008; SSRI, Tokyo, Japan).

RESULTS AND DISCUSSION

We expected that EWH produced by enzymatic treatment from EW would be less allergenic and suppress body fat accumulation in rats. BW gain, food efficiency, intra-abdominal adipose tissue weight, and muscle weight were not significantly different between the C and EW or EWH groups when all groups were provided the same amounts of food (Table 2). No significant differences in BW gain or food intake between the EW and EWH groups were observed, indicating that differences in molecular weight size did not influence the food intake or BW gain.

Fig. 2. Effects of each protein material on viscosity and micellar solubility of TAG and CHO (in vitro). Values are mean±SE \((n=3)\) in each shear velocity point (viscosity test) (A) and mean \((n=2)\) in the micellar solubility test (B).
absorption because EW and EWH can have a higher dispersive activity in vivo. Furthermore, the micellar solubility of CHO and TAG was lower in the EW, but not EWH, compared with the casein group (Fig. 2B).

However, a detailed physicochemical mechanism in the digestive tract was not elucidated in vivo in this study.

Dietary TAG and CHO are absorbed after the micelle formation in the small intestine and transferred to the blood. The serum levels of TAG and total CHO were slightly, but not significantly, lower in the EW and EWH groups than in the C group (Table 2). The fat accumulation in the liver was significantly or slightly lower in the EW and EWH groups than in the C group (Table 3). The SCD indices of the liver were also significantly lower in the EW and EWH groups than in the C group but not significantly different between the EW and EWH groups.

In parallel with the lower fat accumulation in the liver, both hepatic SCD indices were significantly decreased by the dietary EW and EWH. The serum TAG level of rats in the EW and EWH groups was not paralleled by the suppression of hepatic TAG accumulation. According to our previous study carried out under the same conditions except for the ad libitum feeding, dietary EW and EWH significantly both the serum TAG level and hepatic TAG accumulation (5). It has been indicated that the serum TAG level was suppressed to around half by the pair-feeding and lower food intake in comparison with the case of the ad libitum feeding study (5). Down-regulation of hepatic SCD1 expression has been observed through leptin signalling (22). Beppu et al. (22) indicated that suppression of the SCD index and SCD1 expression in the liver occurred in parallel with a lower

### Table 3. Lipid contents and lipid-related enzymatic activities or activity index of the tissues.

|                        | C       | EW      | EWH     |
|------------------------|---------|---------|---------|
| **Liver lipid contents** |         |         |         |
| TAG (mg/g)             | 39.7±3.6| 20.4±1.3***| 24.5±2.2*** |
| CHO (mg/g)             | 4.5±0.3 | 3.5±0.1** | 3.9±0.1(*)  |
| NEFA (µmol/g)          | 17.4±1.9| 13.4±1.4(*) | 12.3±0.9*   |
| **Soleus muscle lipid contents** |         |         |         |
| TAG (mg/g)             | 12.7±1.4| 9.4±1.2(*) | 7.0±0.9**   |
| NEFA (µmol/g)          | 8.4±0.5 | 7.4±0.3  | 8.3±0.4    |
| **Carcass fat analysis** |         |         |         |
| Carcass moisture percentage (%) | 59.0±0.5 | 61.9±0.9** | 60.8±0.6(*) |
| Carcass fat percentage (%) | 15.7±0.7 | 13.6±1.3 | 15.4±0.8    |
| Body fat percentage (%) | 13.3±0.6 | 11.6±1.0 | 13.3±0.8    |
| **Fecal lipids analysis** |         |         |         |
| Food intake (final 3 d) | 35.2±1.9 | 32.7±1.3 | 32.8±1.3    |
| Fecal dry weight (g/3 d) | 2.97±0.24 | 3.16±0.19 | 2.97±0.17   |
| Fecal lipids content (mg/g) | 208.7±9.3 | 241.7±7.9** | 227.4±4.3(*) |
| Fecal TAG content (mg/g) | 1.8±0.1  | 4.7±0.4*** | 4.5±0.4***  |
| Fecal CHO content (mg/g) | 3.5±0.2  | 8.2±0.8*** | 6.9±0.5***  |
| Fecal TBA content (mg/g) | 72.0±5.2 | 84.9±4.8  | 90.6±6.7**  |
| Apparent rate of fat absorption (%) | 94.2±0.3 | 92.2±0.4*** | 93.2±0.2**# |

SCD activity index

|                        | C       | EW      | EWH     |
|------------------------|---------|---------|---------|
| Liver                  |         |         |         |
| C16:1/C16:0            | 0.10±0.0 | 0.06±0.01*** | 0.07±0.01** |
| C18:1/C18:0            | 2.05±0.08 | 1.12±0.08*** | 1.30±0.12*** |
| Soleus muscle          |         |         |         |
| C16:1/C16:0            | 0.14±0.01 | 0.15±0.03 | 0.11±0.01# |
| C18:1/C18:0            | 2.03±0.22 | 1.96±0.10 | 1.71±0.11  |
| Perirenal adipose tissue |         |         |         |
| C16:1/C16:0            | 0.17±0.00 | 0.16±0.02 | 0.17±0.01# |
| C18:1/C18:0            | 6.28±0.00 | 6.43±0.18 | 6.66±0.22  |

G6PDH activity (µmol/min/g tissue)

|                        | C       | EW      | EWH     |
|------------------------|---------|---------|---------|
| Liver                  | 28.6±1.3 | 33.1±1.2* | 31.3±1.5 |
| Soleus muscle          | 1.42±0.08 | 1.40±0.06 | 1.48±0.03 |
| Gastrocnemius muscle   | 0.67±0.04 | 0.64±0.02 | 0.76±0.05# |

FAS activity (µmol/min/g tissue)

|                        | C       | EW      | EWH     |
|------------------------|---------|---------|---------|
| Liver                  | 1.24±0.14 | 1.44±0.18 | 0.99±0.12# |

Values are mean±SE of 6 rats. Statistically significant differences were evaluated by one-way ANOVA and the Fisher-PLSD test. (*), (**), (***) p<0.1, 0.05, 0.01, 0.001, respectively (vs. C group). (#),# p<0.1, 0.05, respectively (vs. EW group).

SCD, stearoyl-CoA desaturase; G6PDH, glucose 6-phosphate dehydrogenase; FAS, fatty acid synthase.
serum leptin level as did the present results and our previous study (5).

TAG accumulation in the soleus muscle was also slightly or significantly lower in the EW and EWH groups than in the C group, although neither SCD index was altered. Matsuoka et al. (12, 17) suggested that EW inhibited lymphatic TAG transport in thoracic lymph duct-cannulated rats, which could reflect the decreases in TAG content in the serum, liver, and soleus muscle in this study. The reasons for the differences in the SCD indices induced by dietary intake of EW and EWH have not been clarified. It was reported that inhibiting SCD1 expression in muscles improved insulin sensitivity by increasing β-oxidation, which can lead to the suppression of obesity (23). To clarify the anti-obesity and anti-T2DM effects of EW and EWH and their regulatory mechanisms, other muscular fat metabolites need to be investigated.

Equicaloric feeding could induce the suppression of ectopic fat accumulation in rats fed the EW and EWH diets because the net absorbed fat was estimated to be lower compared with an ad libitum feeding pattern. Our previous study also showed suppression of the apparent rate of fat absorption by feeding the EW and EWH ad libitum (5). BW gain, carcass fat weight, and intra-abdominal adipose tissue weight were not significantly different among the groups, which may indicate that most of the absorbed fat in each diet was preferentially accumulated in the intra-abdominal adipose tissues and carcass, but not in the liver or muscles. Hepatic G6PDH activity was significantly lower in the C group than in the EW group (Table 3). It has been considered that the suppression of the hepatic G6PDH activity in the C group was owing to the pair-feeding or equicaloric-feeding and repeated fasting periods in the course of a day. It follows that the energy intake of rats in the C group was limited and insufficient to increase fat synthesis in the liver. Ikeda et al. (24) recently demonstrated that long-term fasting and pair-feeding largely influences lipogenesis-related mRNA expression and enzymatic activity in the liver as well as growth and abdominal fat deposition. In this study, collection of blood and tissues was carried out after 12 h of fasting. Therefore, the relationships between fat accumulation and lipogenic pro-

![Fig. 3. Effects of EW and EWH on plasma TAG elevation after the oil loading (C1) and small intestinal transit after the meal administration (C2) in ddY mice (Exp C). Values are mean±SE of 13–15 mice (C1) and 6–9 mice (C2) per group. Statistically significant differences were evaluated by one-way ANOVA and the Fisher-PLSD test. A difference of p<0.05 was considered to be statistically significant.](image-url)
tein expression not only in the liver but also in muscles cannot be precisely explained under the pair-feeding or equicaloric-feeding conditions as it was most suitable to adjust daily energy intake in this study.

We also considered that specific peptides derived from EW can suppress intestinal fat absorption as well as food intake or appetite. Several peptides derived from EW and other food materials suppressed food intake in rodents via several mechanisms (25, 26). It has been suggested that stimulation of gastric hormone secretion by dietary proteins and their physicochemical properties influences gastric emptying time and intestinal absorption of several nutrients (12, 27). Stanstrup et al. (27) recently indicated that whey protein delayed gastric emptying and suppressed the elevation of plasma lipid metabolites. We investigated the effects of EW and EWH in a lipid-induced hypertriglyceridemia dY mouse model in Exp C. Dietary proteins can inhibit pancreatic lipase activity because the amounts of proteins including lipase are increased in the small intestine and the proteins are non-specifically bound to the pancreatic lipase. In this study, we had expected the EW or EWH to disperse widely in the gut and small intestine and to inhibit the pancreatic lipase activity as previously reported (28). In addition, as ovalbumin, a main protein in EW, is resistant to protease and difficult to degrade to smaller molecular proteins, administration of EW or EWH can suppress the lymphatic transport of TAG, lipid-induced hypertriglyceridemia, and TAG levels in the liver and other tissues. However, lipid-induced hypertriglyceridemia was not suppressed by a single administration of EW or EWH, contrary to our expectation (Fig. 3). Nor was the AUC value significantly different (data not shown). Dietary lipid absorption is known to be closely associated with the structure of the small intestinal mucosal membrane. Yamamoto et al. (29) suggested that the length of the villi in the small intestine was associated and parallel with lipid-induced hypertriglyceridemia and the TAG level of the serum and liver in mice. However, it has been indicated that a single administration of the EW and EWH did not change the mucosal structure or its function in the small intestine. It needs to be investigated for longer test periods whether lipid-induced hypertriglyceridemia is suppressed and the mucosal structure and its function are changed or not after the repeated administration or chronic intake of EW-derived proteins.

The meal transit rate in the small intestine can be associated with the food intake and fat absorption and is often used to evaluate the suppressive effects on food intake, appetite, and digestion (14, 26). However, like the results of intestinal fat absorption, the meal transit was not suppressed by a single administration of the EW or EWH (Fig. 3). Matsuoka et al. (17) showed the CHO-lowering effects and inhibition of the micellar CHO solubility of the EW in rats for 3 wk. Although detailed mechanisms have not been clarified, chronic intake, but not a single administration, of EW or EW-derived proteins may be important to suppress fat absorption in the small intestine.

The significant or slight decrease in fasting serum levels of glucose and ALP in the EW and EWH groups might also be associated with inhibition of the digestion of dietary nutrients by the dietary EW-derived proteins (5, 28). This is almost consistent with our previous results in rats fed ad libitum (4, 5). Our previous study (4) also showed that EWH decreased fasting serum ALP levels in a T2DM rat model. Small-intestinal ALP activity is increased when dietary lipids are absorbed and transferred from the small intestine to intestinal lymph (30), indicating that decreased serum ALP activity might down-regulate the intestinal digestion and absorption of lipids and carbohydrates. As l-phenylalanine is a small-intestinal ALP inhibitor (31), the higher content of phenylalanine in the EW and EWH (5.7% and 6.0%, respectively; data not shown) compared with the casein (4.8%, data not shown) diet may cause the lower serum ALP activity and apparent rate of lipid absorption in the EW and EWH groups. In our unpublished data, dietary intake of EW and/or EWH for 8 wk also improved serum glucose levels on glucose tolerance and insulin tolerance tests in T2DM mice. However, the HOMA-IR and HOMA-β were not significantly different among the groups in this study, perhaps owing to a difference in the animal model used. To clarify the reasons that dietary EW and/or EWH decrease fasting serum glucose levels, intestinal enzymes related to digestion and absorption of carbohydrate and glucose uptake in several tissues should be investigated.

In summary, equicaloric dietary intake of EW-derived protein increased the fecal lipid excretion and suppressed the serum leptin levels and dietary fat absorption, and it could suppress the accumulation of lipids in the liver and muscles. However, there were no suppressive effects of dietary EW or EWH on fat accumulation in the intra-abdominal adipose tissues, carcass, or total body of rats. Viscosity was suppressed by the EW and EWH, and micellar solubility was suppressed by the EW, indicating that EW-derived protein has a higher dispersity in digestive tracts. Possible mechanisms for the suppression of ectopic fat accumulation were investigated, but a single administration of EW or EWH did not suppress the lipid-induced hypertriglyceridemia nor delay the rate of small-intestinal food transit in mice. In conclusion, the EW-derived low-allergic protein material can suppress intestinal fat absorption and ectopic fat accumulation.

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REFERENCES

1) Stengel A, Goebel-Stengel M, Wang L, Hu E, Karasawa H, Pisegna JR, Tache Y. 2013. High-protein diet selectively reduces fat mass and improves glucose tolerance in Western-type diet-induced obese rats. Am J Physiol
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2) Mine Y. 1995. Recent advances in the understanding of egg white protein functionality. *Trends Food Sci Technol* **6**: 225–232.

3) Yu Z, Yin Y, Zhao W, Yu Y, Liu B, Liu J, Chen F. 2011. Novel peptides derived from egg white protein inhibiting alpha-glucosidase. *Food Chem* **129**: 1376–1382.

4) Ochiai M, Kuroda T, Matsuo T. 2014. Increased muscular triglyceride content and hyperglycemia in Goto-Kakizaki rat are decreased by egg white hydrolysate. *Int J Food Sci Nutr* **65**: 495–501.

5) Ochiai M, Matsuo T. 2014. Effect of egg white and its hydrolysate on stearyo-CoA desaturase index and fat accumulation in rat tissues. *Int J Food Sci Nutr* **65**: 948–952.

6) Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. 1985. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentration in man. *Diabetologia* **28**: 412–419.

7) Folch J, Lees M, Sloan Stanley GH. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* **226**: 497–509.

8) Mickelsen O, Anderson AA. 1959. A method for preparing intact animals for carcass analyses. *J Lab Clin Med* **53**: 282–290.

9) De Bont AJ, Rombos DR, Tsai AC, Waterman RA, Leveille GA. 1975. Influence of alterations in meal frequency on lipogenesis and body fat content in the rat. *Proc Soc Exp Biol Med* **149**: 849–854.

10) Kelley DS, Kletzen RF. 1984. Ethanol modulation of the hormonal and nutritional regulation of glucose 6-phosphate dehydrogenase activity in primary cultures of rat hepatocytes. *Biochem J* **217**: 543–549.

11) Nepokroeff CM, Lakshmanan MR, Porter JW. 1984. Inhibition of pancreatic and microbial lipases by alpha-glucosidase. *Food Chem* **129**: 1376–1382.

12) Ochiai M, Kuroda T, Matsuo T. 2014. Increased muscular triglyceride content and hyperglycemia in Goto-Kakizaki rat are decreased by egg white hydrolysate. *Int J Food Sci Nutr* **65**: 495–501.

13) Yamazaki T, Kishimoto K, Etsuki O. 2012. The diD mouse: a model of postprandial hypertriglyceridemia in response to dietary fat. *J Lipid Res* **53**: 2024–2037.

14) Kagebayashi T, Kontani N, Yamada Y, Minatsuzhi T, Arai T, Kino K, Ohinata K. 2012. Novel CCK-dependent vasorelaxing dipeptide, Arg-Phe, decreases blood pressure and food intake in rodents. *Mol Nutr Food Res* **56**: 1456–1463.

15) Klok MD, Jakobsdottir S, Drent ML. 2007. The role of leptin and ghrelin in the regulation of food intake and body weight in humans: a review. *Obes Rev* **8**: 21–34.

16) Prieto X, Tung YC, Griffin JL, Farooqi IS, O’Rahilly S, Coll AP. 2008. Leptin regulates peripheral lipid metabolism primarily through central effects on food intake. *Endocrinology* **149**: 5432–5439.

17) Matsuoka R, Kimura M, Muto A, Masuda Y, Sato M, Imaiuzumi K. 2008. Mechanism for the cholesterol-lowering action of egg white protein in rats. *Biosci Biotechnol Biochem* **72**: 1506–1512.

18) Wang J, Shimada M, Kato Y, Kusada M, Nagaoka S. 2015. Cholesterol-lowering effect of rice bran protein containing bile acid-binding proteins. *Biose Biotechnol Biochem* **79**: 456–461.

19) Hosomi R, Fukunaga K, Arai H, Kanda S, Nishiyama T, Yoshida M. 2011. Fish protein decreases serum cholesterol in rats by inhibition of cholesterol and bile acid absorption. *J Food Sci* **76**: H116–H121.

20) Kobayashi M, Nishizawa M, Inoue N, Hosoya T, Yoshida M, Ukawa Y, Sagesuka YM, Doi T, Nakayama T, Kuzumazawa S. Ikeda I. 2014. Epigallocatechin gallate decreases the micellar solubility of cholesterol via specific interaction with phosphatidylcholine. *J Agric Food Chem* **62**: 2881–2890.

21) Shirouuchi B, Kawamura S, Matsuoka R, Baba S, Nagata K, Shiratake S, Tomoyori H, Imaiuzumi K, Sato M. 2011. Dietary guar gum reduces lymph flow and diminishes lipid transport in thoracic duct-cannulated rats. *Lipids* **46**: 789–793.

22) Beppu F, Hosokawa M, Yim MJ, Shinoda T, Miyashita K. 2013. Down-regulation of hepatic stearyo-CoA desaturase-1 expression by fucocoxanthin via leptin signaling in diabetic/obese KK-Aty mice. *Lipids* **48**: 449–455.

23) Kobayashi M, Kuroda T, Matsuo T. 2014. Increased muscular triglyceride content and hyperglycemia in Goto-Kakizaki rat are decreased by egg white hydrolysate. *Int J Food Sci Nutr* **65**: 495–501.

24) Ikeda I, Metoki K, Yamahira T, Kato M, Inoue N, Nagao K, Yamagita T, Shirakawa H, Komai M. 2014. Impact of lasting time on hepatic lipid metabolism in nutritional animal studies. *Biosci Biotechnol Biochem* **78**: 1584–1591.

25) Ohinata K, Fujiwata Y, Shingo F, Masatsugu H, Yoshikawa M. 2009. Orally administered nodokinin, an angiotensin AT2 receptor agonist, suppresses food intake via prostaglandin E2-dependent mechanism in mice. *Peptides* **30**: 1105–1108.

26) Kaneko K, Iwasaki M, Yoshikawa M, Ohinata K. 2010. Orally administered somorphins, soy-derived opioid peptides, suppress feeding and intestinal transit via gut mu(1)-receptor coupled to 5-HT(1A), D(2), and GABA(B) systems. *Am J Physiol Endocrinol Metab* **288**: E599–E607.

27) Stanstrup J, Schou SS, Holmer-Jensen J, Hermansen K, Dragsted LO. 2014. Whey protein delays gastric emptying and suppresses plasma fatty acids and their metabolites compared to casein, gluten, and fish protein. *J Proteome Res* **13**: 2396–2408.

28) Gargouri Y, Julien R, Sugihara A, Verger R, Sarda L. 1984. Inhibition of pancreatic and microbial lipases by proteins. *Biochim Biophys Acta* **795**: 326–331.

29) Yamamoto K, Shuang E, Hatakeyama Y, Sakamoto Y, Tsuduki T. 2015. High-fat diet intake from senescence primarily through central effects on food intake and body weight in humans: a review. *Obes Rev* **8**: 21–34.

30) Flock EV, Bollman JL. 1948. Alkaline phosphatase in the small intestine, and increasing beta-oxidation in skeletal muscle. *J Physiol Endocrinol Metab* **288**: E599–E607.

31) Beppu F, Hosokawa M, Yim MJ, Shinoda T, Miyashita K. 2013. Down-regulation of hepatic stearyo-CoA desaturase-1 expression by fucocoxanthin via leptin signaling in diabetic/obese KK-Aty mice. *Lipids* **48**: 449–455.

32) Dobrzyn A, Dobrzyn P, Lee SH, Miyazaki M, Cohen P, Aslilmaz E, Hardie DG, Friedman JM, Niambi JM. 2005. Stearyo-CoA desaturase-1 deficiency reduces ceramide synthesis by downregulating serine palmitoyltransferase and increasing beta-oxidation in skeletal muscle. *Am J Physiol Endocrinol Metab* **288**: E599–E607.

33) Ikeda I, Metoki K, Yamahira T, Kato M, Inoue N, Nagao K, Yamagita T, Shirakawa H, Komai M. 2014. Impact of lasting time on hepatic lipid metabolism in nutritional animal studies. *Biosci Biotechnol Biochem* **78**: 1584–1591.

34) Ohinata K, Fujiwata Y, Shingo F, Masatsugu H, Yoshikawa M. 2009. Orally administered nodokinin, an angiotensin AT2 receptor agonist, suppresses food intake via prostaglandin E2-dependent mechanism in mice. *Peptides* **30**: 1105–1108.

35) Kaneko K, Iwasaki M, Yoshikawa M, Ohinata K. 2010. Orally administered somorphins, soy-derived opioid peptides, suppress feeding and intestinal transit via gut mu(1)-receptor coupled to 5-HT(1A), D(2), and GABA(B) systems. *Am J Physiol Gastrointest Liver Physiol* **299**: G799–G805.

36) Stanstrup J, Schou SS, Holmer-Jensen J, Hermansen K, Dragsted LO. 2014. Whey protein delays gastric emptying and suppresses plasma fatty acids and their metabolites compared to casein, gluten, and fish protein. *J Proteome Res* **13**: 2396–2408.

37) Gargouri Y, Julien R, Sugihara A, Verger R, Sarda L. 1984. Inhibition of pancreatic and microbial lipases by proteins. *Biochim Biophys Acta* **795**: 326–331.