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REVIEW

Alleviation of Metabolic Syndrome with Dietary Egg White Protein

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Abstract: Abdominal fat accumulation causes metabolic syndrome, which is a cluster of metabolic abnormalities such as dyslipidemia, glucose intolerance, insulin resistance or hyperinsulinemia, and hypertension, leading to the development of diabetes and cardiovascular disease. Diets are known to contribute to the development or prevention of metabolic syndrome. Several studies have reported that the quality of dietary proteins may be an important modulator of the risk of this syndrome. We investigated the effects of consuming egg white protein (EWP) or lactic-fermented egg white (LE), an easy-to-consume form of egg white, on the development of metabolic syndrome in animal models and humans. In comparison with casein, dietary EWP decreased lymphatic lipid transport in thoracic lymph duct-cannulated rats. In an *in vitro* experiment, EWP pepsin hydrolysate decreased the cholesterol micellar solubility and cholesterol transfer rate from micelles to oil phase, and increased water-holding capacity, settling volume in water, and relative viscosity compared with casein pepsin hydrolysate. The daily consumption of LE for 8 weeks reduced serum total cholesterol and LDL cholesterol levels in men with mild hypercholesterolemia. Furthermore, dietary EWP reduced the body fat mass of rats by increasing the body protein mass and accelerating hepatic β-oxidation. The daily consumption of LE for 12 weeks reduced the visceral fat area and improved the ratio of the visceral to subcutaneous fat area. Taken together, these results indicated that dietary EWP and LE would be useful for preventing or alleviating metabolic syndrome.

Key words: egg white protein, lactic-fermented egg white, anti-obesity effect, lipid-lowering effect, metabolic syndrome

1 Introduction

Lifestyle changes, such as overnutrition and lack of exercise, have increased the prevalence of lifestyle-related diseases including obesity, dyslipidemia, diabetes, hypertension, and arteriosclerosis. Metabolic syndrome is a cluster of metabolic abnormalities including abdominal obesity, dyslipidemia, impaired fasting glucose, and high blood pressure, which can lead to the development of diabetes and cardiovascular disease[1]. The prevalence of metabolic syndrome is estimated to be approximately 20–25%[2, 3]. In Japan, one in two men and one in five women aged 40–74 years have metabolic syndrome or pre-metabolic syndrome (abdominal obesity with one metabolic abnormality), which is becoming a serious problem[4]. As abdominal obesity can contribute to metabolic syndrome, it is critical to attain and maintain a healthy body weight. The treatment or prevention of obesity requires a negative energy balance in the body, which is most effectively achieved with an energy-restricted diet[5]. However, this approach results in increased feelings of hunger and appetite, leading to the development of appetite disorders. Body weight loss involves the reduction of fat mass and lean body mass. Nevertheless, body weight loss should be accompanied by a reduction in energy intake without affecting appetite and the maintenance of energy expenditure with a constant lean body mass. Diets are known to contribute to the development or prevention of metabolic syndrome. Several studies have reported that the quality of dietary proteins may be an important modulator of the risk of this syndrome[6–12].

Dietary proteins are commonly categorized according to their essential amino acid content and basic taxonomy

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(plant-based and animal-based proteins)[13]. Several plant foods such as nuts, seeds, legumes, and grains contain high protein contents. Except for soybeans, most plant proteins are limited in one or more essential amino acids[13, 14]. On the other hand, most animal-based proteins contain adequate proportions of all the essential amino acids necessary for human nutrition[13, 14]. Among animal-based proteins, egg whites are a fat-free, high-protein food. Egg white protein (EWP) contains many essential amino acids, having an amino acid score of 100. EWP is a high-quality source of protein compared with proteins derived from milk and soybeans[15]. In addition, compared with these proteins and other proteins with an amino acid score of 100, EWP has a higher net protein utilization[16]. Therefore, we investigated the physiological functions of dietary EWP. This review describes the effects of dietary EWP on metabolic syndrome.

2 Effects of dietary EWP on lipid metabolism in metabolic syndrome

2.1 Effects of dietary EWP on the lipid metabolism of rats

Our previous study showed that compared with casein, dietary EWP decreased serum and lipid cholesterol levels and increased fecal sterol excretion in rats[17]. However, the mechanism of the cholesterol-lowering effect of EWP remains unclear. Moreover, previous studies have not evaluated the effects of EWP on lymphatic lipid transport. Therefore, we investigated the effects of dietary EWP on lymphatic lipid transport in thoracic lymph duct-cannulated rats. Figure 1 shows the lymphatic transport of cholesterol and triacylglycerol (TAG) in thoracic lymph duct-cannulated rats[18]. We found that dietary EWP decreased the intestinal absorption of cholesterol and TAG in the rats. These results are consistent with the serum and hepatic cholesterol-lowering action of EWP observed in our previous study[17]. Most dietary lipids are absorbed in the proximal small intestine (jejunum) and subsequently transported by the lymphatic system. We hypothesized that EWP pepsin hydrolysate (EWP-ph) in the stomach may play a major role in decreasing lipid absorption. To investigate the hypothesis, we examined the degree of hydrolysis of casein and EWP by pepsin[18]. As shown in Fig. 2, casein was almost digested by pepsin. On the other hand, two bands were observed for EWP-ph. These bands were identified as ovalbumin (45 kDa) and lysozyme (14.3 kDa). EWP contains 54% ovalbumin, 13% ovotransferrin, 11% ovomucoid, and 3.5% lysozyme[19]. Therefore, ovalbumin is the most abundant protein in EWP. In our study, it was almost resistant to digestion by pepsin. Next, we prepared pepsin hydrolysates of EWP and its constituent fractions and assessed physicochemical properties including micellar formation[18]. The results showed that the pepsin hydrolysates of EWP, ovalbumin, and ovotransferrin inhibited the micellar solubility of cholesterol (Fig. 3A). Taken together with the results of SDS-PAGE (Fig. 2), ovalbumin may be responsible for the cholesterol-lowering activity of EWP. In addition, EWP-ph significantly inhibited cholesterol transfer from micelles to oil phase, indicating the inhibition of cholesterol monomer release from micelles (Fig. 3B).

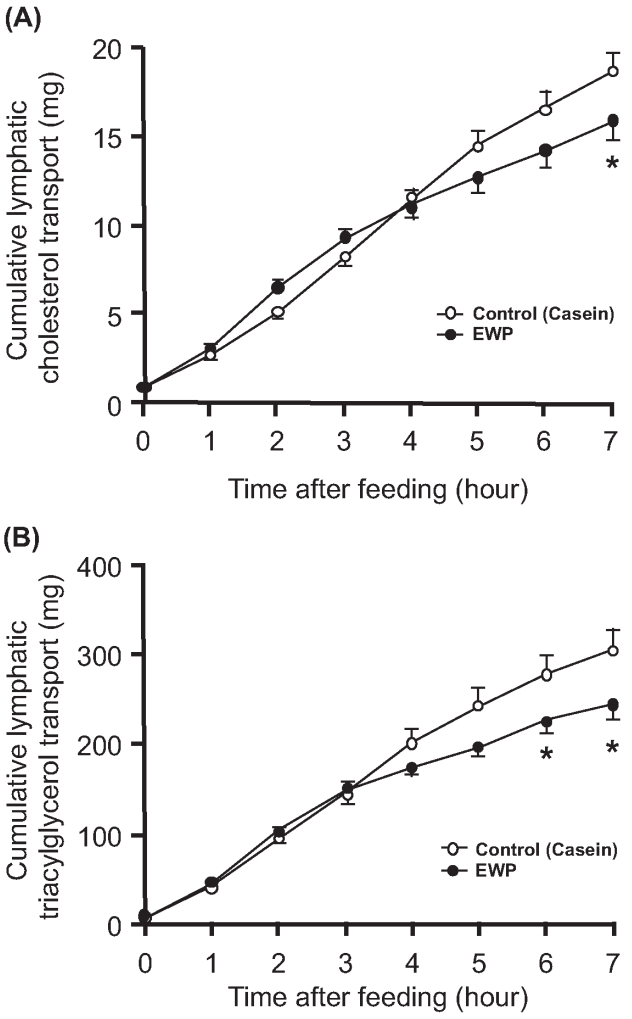

Fig. 1 Cumulative lymphatic transport of (A) cholesterol and (B) triacylglycerol in rats fed diets containing 20% casein or 20% EWP. Values are expressed as the mean ± SEM (n = 6/group). The asterisk indicates a significant difference between the two groups (*$p < 0.05$). These figures are reconstructed from the figure in the reference 18. Please refer to the reference 18 regarding detailed experimental condition.

We found that dietary EWP decreased lymphatic TAG transport in thoracic lymph duct-cannulated rats (Fig. 1B). This finding is consistent with the decreased serum and hepatic TAG levels observed in our previous study, which were 26% and 37%, respectively, in rats fed a EWP diet for 3 weeks[17]. Ovalbumin has been reported to exhibit

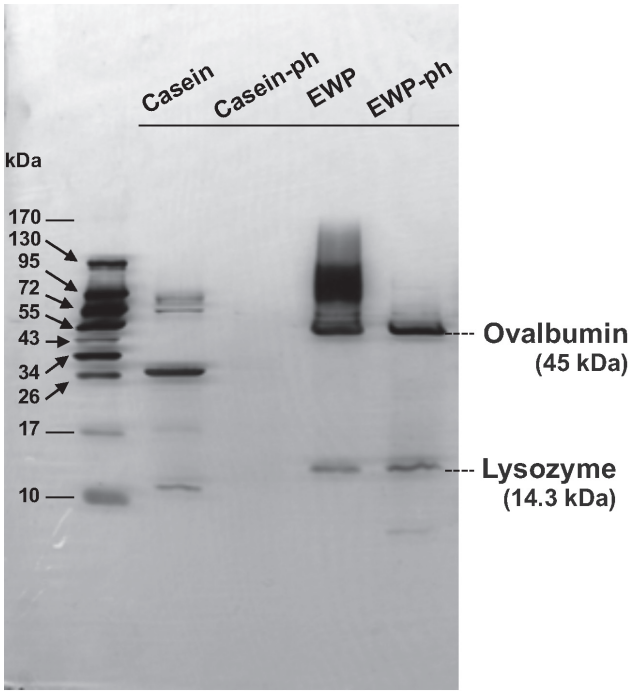

Fig. 2 Electrophoresis of casein, EWP, and pepsin hydrolysates. The degree of hydrolysis of casein and EWP was determined by SDS-PAGE with silver staining. This figure is reconstructed from the figure in the reference 18. Please refer to the reference 18 regarding detailed experimental condition.

pancreatic lipase inhibitory activity[20]. Ovalbumin is not digested by pepsin in the stomach because of its protease inhibitory action; it reaches the intestine and acts as a lipase inhibitor. Therefore, dietary EWP could decrease the intestinal absorption and lymphatic transport of TAG, leading to a decrease in serum and hepatic TAG levels.

Physicochemical properties such as the water-holding capacity (WHC), settling volume in water (SV), and relative viscosity of casein pepsin hydrolysate (casein-ph) and EWP-ph were evaluated. There was no significant difference in the WHC between casein and EWP. However, the WHC of EWP-ph was significantly higher than that of casein-ph (casein-ph, 0.32 ± 0.09; EWP-ph, 2.11 ± 0.09; wet wt (g)/dry wt (g); $p < 0.05$). In agreement with WHC results, the SV and relative viscosity of EWP-ph were significantly higher than those of casein-ph (data not shown; please refer to the reference 18). These characteristics of EWP-ph are similar to those of water-soluble fibers such as guar gum[21]. Under alkaline conditions, fatty acids, water, and EWP combine in the intestine after ingestion, and EWP turns into a gel because of its physicochemical properties[22–26]. Based on the findings, the gel form of EWP may hold micelles. Furthermore, the gel mixture containing EWP, fatty acids, cholesterol, and water may contribute to the inhibition of lipid absorption. Taken together, dietary

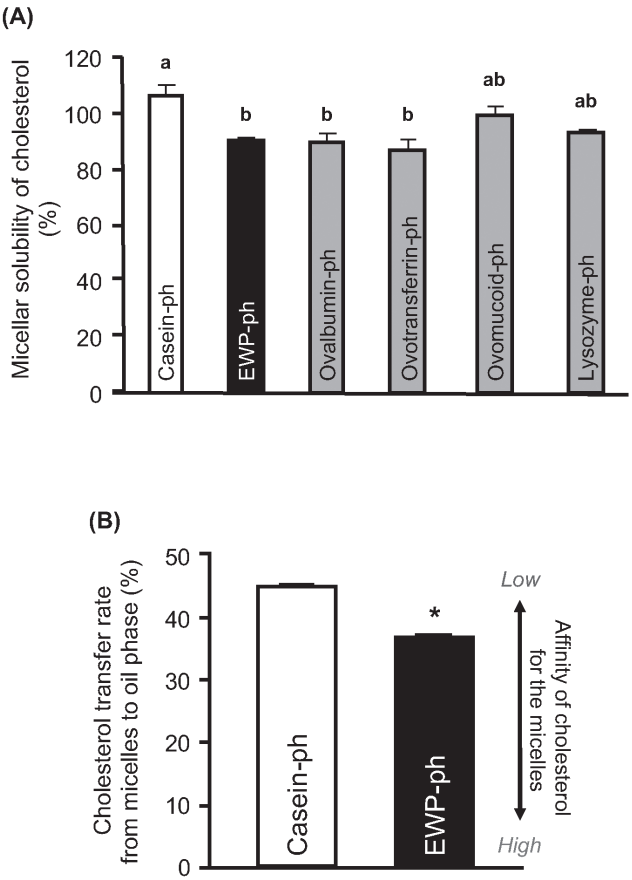

Fig. 3 (A) Effects of EWP and its constituent protein hydrolysates on the micellar solubility of cholesterol *in vitro*. Values were calculated using the following formula: (micellar cholesterol content with protein / micellar cholesterol content without protein) × 100. Values are expressed as the mean ± SEM of three samples. Different letters show a significant difference at $p < 0.05$. (B) Effects of EWP-ph on the transfer of micellar cholesterol to triolein *in vitro*. Values were calculated using the following formula: (cholesterol content in the triolein phase / original micellar cholesterol content) × 100. Values are expressed as the mean ± SEM of three samples. The asterisk indicates a significant difference between the two groups (*$p < 0.05$). These figures are reconstructed from the figure in the reference 18. Please refer to the reference 18 regarding detailed experimental condition.

EWP could decrease intestinal lipid absorption through a combination of physicochemical effects in the gut.

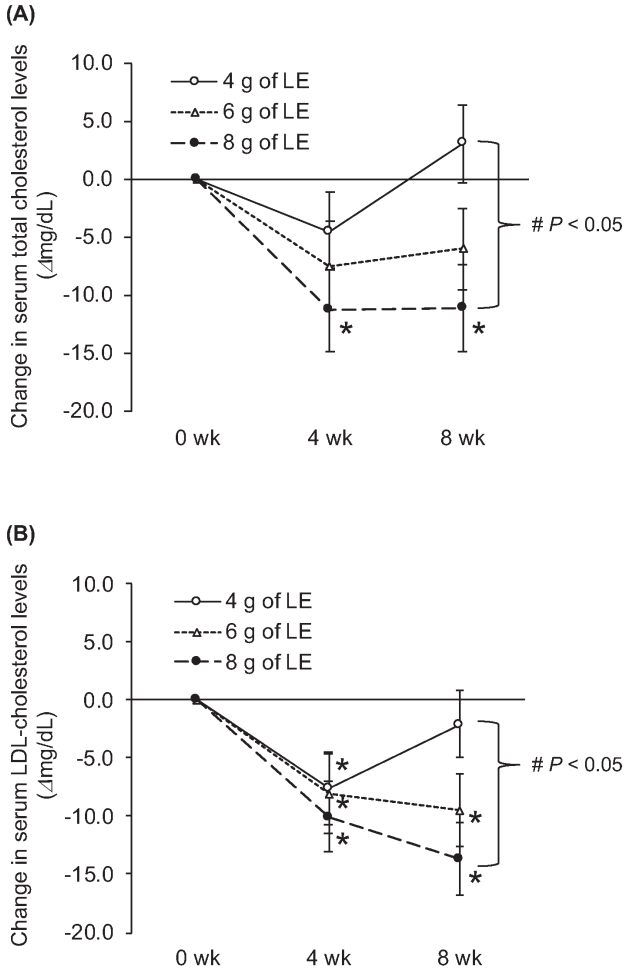

Fig. 4 Changes in the serum (A) total cholesterol levels and (B) LDL cholesterol levels of subjects with mild hypercholesterolemia who consumed LE drinks containing 4, 6, or 8 g of protein daily for 8 weeks. Values are expressed as the mean ± SEM (4 g group, n = 28; 6 g group, n = 31; 8 g group, n = 29). *$p < 0.05$ versus 0 week, #$p < 0.05$ versus the 4 g group. These figures are reconstructed from the figure in the reference 29. Please refer to the reference 29 regarding detailed experimental condition.

2.2 Effects of lactic-fermented egg white (LE) on the serum cholesterol levels of Japanese subjects with mild hypercholesterolemia

As mentioned previously, EWP could exert lipid-lowering effects by inhibiting lipid absorption in the intestine. Asato *et al.* conducted an intervention study in which female university students with moderate hypercholesterolemia consumed 23 g of protein (cheese, tofu, or EWP) over 30 days[27]. In comparison with cheese, EWP significantly reduced serum total cholesterol and LDL cholesterol levels to an extent comparable to those following tofu (soybean protein) consumption. However, the use of egg white is challenging in dietary therapy because of its hydrogen sulfide odor. To address this issue, lactic-fermented egg white (LE) has been developed[28]. LE is easier to consume because the hydrogen sulfide level of LE is lower than that of egg white. Ovalbumin and lysozyme in egg white are stable after the fermentation treatment (data not shown). To evaluate the beneficial effects of dietary EWP through the consumption of LE, we attempted to validate the extrapolation of animal-derived data to humans. In a double-blind, parallel-arm study involving 88 Japanese adult men (aged 20–65 years) with mild hypercholesterolemia (serum total cholesterol level ≥ 200 mg/dL), the effects of daily intake of LE on serum cholesterol levels were evaluated[29]. The subjects consumed LE drinks containing 4, 6, or 8 g of protein daily for 8 weeks. No abnormal increases or decreases in hematological parameters and liver and kidney function indices were observed (data not shown; please refer to the reference 29). The consumption of LE for 8 weeks at a daily dose of 8 g reduced serum total cholesterol (Fig. 4A) and LDL cholesterol (Fig. 4B) levels in the subjects, suggesting that this may be effective in preventing arteriosclerotic diseases.

3 Physiological effects of dietary EWP on obesity in metabolic syndrome

3.1 Effects of dietary EWP on the body fat mass of rats

Most studies reporting a decrease in abdominal fat following the consumption of functional foods have identified bioactive components that contribute to this reduction. The consumption of lactoferrin, a protein fraction in milk, has been found to decrease abdominal fat in obese subjects[30]. In addition, the consumption of β-conglycinin, a soybean protein fraction, has been reported to decrease abdominal fat in subjects with a body mass index (BMI) of 25–30 (waist size of more than 85 cm)[31]. Therefore, protein consumption can potentially lead to a decrease in abdominal fat. However, the effects of dietary EWP on body fat mass remain unclear. Therefore, we investigated the effects of dietary EWP on the body composition of rats[32]. Male Sprague-Dawley rats were fed diets containing 20% casein or EWP for 4 weeks. The carcass protein content of the EWP group was significantly higher than that of the casein group (casein group, 18.6 ± 0.2; EWP group, 20.9 ± 0.5; g/100 g of body weight; $p < 0.05$). On the other hand, the carcass TAG (fat) content of the EWP group was significantly lower than that of the casein group (casein group, 9.57 ± 0.72; EWP group, 7.18 ± 0.63; g/100 g of body weight; $p < 0.05$). Based on the results, we also evaluated the effects of dietary EWP on the abdominal fat mass and adipocyte size of rats[32]. The abdominal white adipose tissue weights (perirenal + retroperitoneal and subcutaneous) were significantly decreased in the EWP group

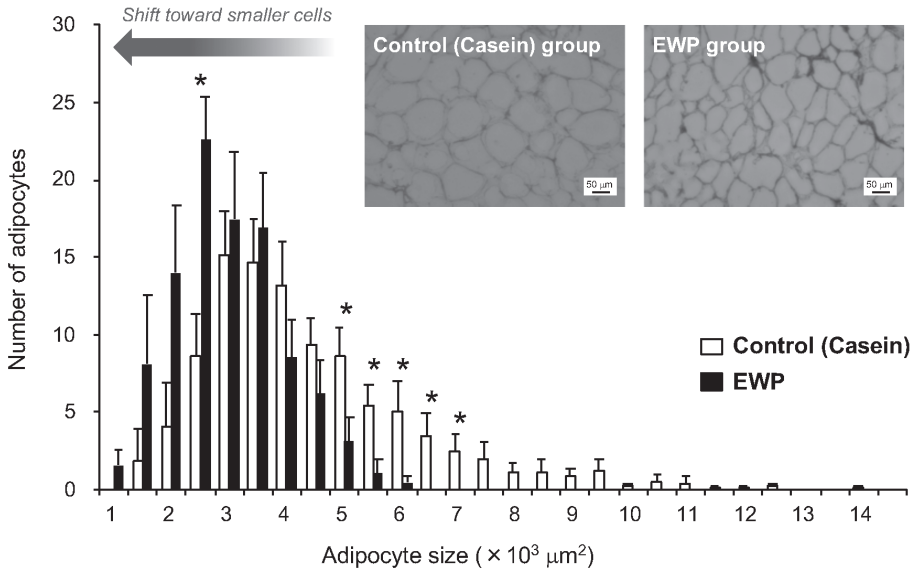

Fig. 5 Histograms of the adipocyte sizes of rats fed diets containing 20% casein or 20% EWP. Values are expressed as the mean ± SEM (casein group, n = 10; EWP group, n = 9). The asterisk indicates a significant difference between the two groups (*$p < 0.05$). This figure is reconstructed from the figure in the reference 32. Please refer to the reference 32 regarding detailed experimental condition.

(data not shown; please refer to the reference 32). The average adipocyte size in perirenal + retroperitoneal white adipose tissues was significantly smaller in the EWP group than in the casein group. As shown in Fig. 5, the number of small adipocytes was increased, whereas the number of large adipocytes was decreased in the EWP group. In this study, dietary EWP also significantly decreased serum and hepatic TAG levels and enhanced activities of carnitine palmitoyltransferase (a key enzyme in mitochondrial fatty acid β-oxidation) and acyl-CoA oxidase (the initial enzyme of the peroxisomal β-oxidation system) in the liver of rats (data not shown; please refer to the reference 32). Our results indicated that in addition to the inhibition of dietary TAG absorption reported previously[18], dietary EWP could reduce the body fat mass of rats by increasing the body protein mass and accelerating hepatic β-oxidation. Yamada *et al.* showed that soymorphin-5 (YPFVV), a soy-derived μ-opioid peptide, enhanced hepatic β-oxidation through activating PPARα systems in diabetic KKAy mice[33]. Therefore, it is possible that EWP also contains bioactive peptides. In the future, it will be necessary to examine how dietary EWP regulates hepatic β-oxidation, using gene analysis or other methods.

Although we did not use animal models with severe obesity and glucose intolerance, dietary EWP reduced serum leptin levels as reflected by the reduction of body fat mass in rats[32]. Circulating leptin levels positively correlates with insulin resistance in subjects[34, 35]. Therefore, anti-obesity effect of dietary EWP may contribute to prevention or alleviation of glucose intolerance. In support of our opinion, other research group showed that oral administration of EWP-ph reduced abdominal fat mass and plasma glucose levels in high-fat/high-dextrose diet-induced metabolic syndrome model rats[36], and that the administration reduced plasma insulin levels and improved pancreatic β-cell functionality in obese Zucker rats[37].

3.2 Effects of LE on the visceral fat mass of Japanese subjects with visceral fat obesity

In the experiment with rats, EWP was found to exert anti-obesity effects by inhibiting dietary TAG absorption, increasing the body protein mass, and enhancing hepatic β-oxidation. Therefore, in a double-blind, placebo-controlled study involving 37 Japanese adults (aged ≥ 40 years) with visceral fat obesity [visceral fat area (VFA) ≥ 100 cm^2], the effects of daily intake of LE on the VFA were evaluated[38]. The subjects were randomly divided into two groups (control and LE). The control group was given a drink containing whey (8 g of protein), and the LE group was given a drink containing LE (8 g of protein) every day for 12 weeks. No abnormal increases or decreases in hematological parameters and liver and kidney function indices were observed, suggesting that EWP may be safe for consumption (data not shown; please refer to the reference 38). As shown in Fig. 6A–6C, the consumption of LE for 12 weeks at a daily dose of 8 g reduced the VFA and improved the ratio of the visceral to subcutaneous fat area, leading to a decreased risk of metabolic syndrome.

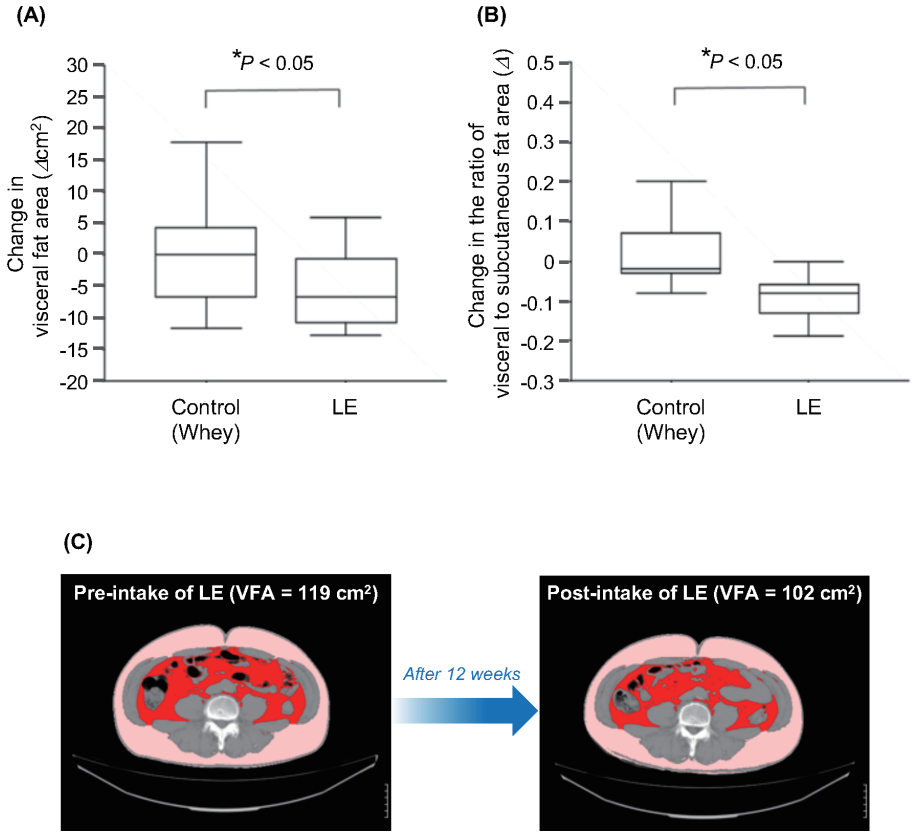

Fig. 6 Changes in the (A) visceral fat area and (B) ratio of the visceral to subcutaneous fat area of subjects with visceral fat obesity who consumed whey drink (8 g of protein) or LE drink (8 g of protein) daily for 12 weeks. Values are expressed as box plots; the center line of each box represents the median. (C) A photographic example of the change in visceral fat area of the subjects who consumed LE drink (8 g of protein) daily for 12 weeks (red, visceral fat; pink, subcutaneous fat). Values are expressed as the mean ± SEM (control group, n = 18; LE group, n = 19). The asterisk indicates a significant difference between the two groups (*$p < 0.05$). These figures are reconstructed from the figure in the reference 38. Please refer to the reference 38 regarding detailed experimental condition.

4 Conclusion

This review describes the physiological effects of dietary EWP on the development of metabolic syndrome. Experimental studies using rats have demonstrated that dietary EWP could reduce the body fat mass of rats by inhibiting dietary TAG absorption, accelerating fatty acid β-oxidation in the liver, and increasing the body protein mass. In addition, dietary EWP could inhibit dietary cholesterol absorption by restricting the passage of cholesterol into and out of micelles and increasing the WHC, SV, and relative viscosity, leading to decreased serum cholesterol levels. In agreement with the results, human intervention studies have also demonstrated that the consumption of LE could improve visceral fat obesity and reduce serum total cholesterol and LDL cholesterol levels. Therefore, the consumption of EWP could improve the quality of life of patients with metabolic syndrome. Furthermore, if LE, which facilitates egg white intake, may be produced commercially for consumption, it could provide a good source of protein and reduce visceral fat and/or prevent metabolic syndrome; this would be beneficial for promoting a healthy lifestyle among Japanese people.

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Conflicts of interest

R.M. is an employee of Kewpie Corporation. There are no other patents, products in development, or marketed products to declare. B.S. has no conflicts of interest to declare.

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