Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/jff

Effects of desalted duck egg white peptides and their products on calcium absorption in rats

Ningning Zhao, Jun Hu, Tao Hou, Zhili Ma, Chi Wang, Hui He *

College of Food Science and Technology, Huazhong Agricultural University, Wuhan 430070, China

ARTICLE INFO

Article history:
Received 26 February 2014
Received in revised form 26 March 2014
Accepted 26 March 2014
Available online 14 April 2014

Keywords:
Duck egg white peptides (DPs)
Phosphorylation
Peptides–calcium complexes
Calcium absorption
Bone

ABSTRACT

The effects of desalted duck egg white peptides (DPs), phosphorylated DPs (PDPs) and DPs–calcium complexes (DPs-Ca) on calcium absorption *in vivo* were investigated. Ninety fast-growing male rats were divided into 10 groups and treated with different dosages of $CaCO_3$, calcium gluconate, DPs-Ca as well as CPPs, DPs and PDPs supplemented with $CaCO_3$. Eight-week oral administration results indicated that the high dosage PDPs+$CaCO_3$ group was significantly higher than the high dosage DPs+$CaCO_3$ group in calcium absorption, bone calcium content, BMD and maximum stress, but lower in ALP activity ($P < 0.05$). Additionally, the high dosage DPs-Ca group showed higher bone indices than the high dosage DPs+$CaCO_3$, calcium gluconate and $CaCO_3$ groups ($P < 0.05$). These results suggest that *in vivo*, phosphorylated DPs are more effective than DPs in improving calcium absorption and bone strength, and DPs–calcium complexes are more beneficial to bone tissues than the DPs and $CaCO_3$ mixture.

1. Introduction

Calcium plays a central role in any discussion of bone health. A low intake and bioavailability of calcium may affect peak bone mass negatively (Cashman, 2002). However, most Chinese have an insufficient intake of milk products, and some of the green vegetables have a low bioavailability of calcium. Weakly binding calcium to some certain substances (especially protein and peptides) to prevent calcium from precipitating can effectively increase the calcium absorption in the body (Daengprok et al., 2003). It has been reported that the substances like casein phosphopeptides (CPPs) and phosvitin phosphopeptides (PPPs) can increase the intestinal calcium absorption via the phosphate residues of serine, which can be chelated with calcium to form soluble and stable complexes (Jiang & Mine, 2001; Sato, Noguchi, & Naito, 1986). Additionally, soybean peptides, without phosphorylated residues, have also been discovered to bind calcium by the carboxyl groups of Glu and Asp residues (Bao, Lv, Yang, Ren, & Guo, 2008; Kumagai et al., 2004). After that, the peptides from fish bone (Jung & Kim, 2007; Jung, Lee, & Kim, 2006), shrimp processing by-products (Huang, Ren, & Jiang, 2011), and whey protein (Pan, Lu, & Zeng, 2013) were also found to promote the absorption of calcium whether *in vivo* or *in vitro*. Our previous work demonstrated that the peptides from hen egg white (EPs) could also improve the bone mineral densities in rats (Han, 2011).

Salted duck egg is one of the most popular and traditional preserved egg products in both Southeast Asia and China, and it contains all essential amino acids for human beings (Kaewmanee, Benjakul, & Visessanguan, 2009, 2011). In addition to being eaten as the whole egg, salted egg yolk is widely used as fillings in foods such as glutinous rice dumplings and moon cakes (Kaewmanee, Benjakul, & Visessanguan, 2012). Due to the content of 7–10% sodium chloride, large amounts of salted duck egg white are discarded every year. After hydrolysis by

* *Corresponding author.* Tel.: +86 27 87280346; fax: +86 27 87282966.
E-mail address: hehui@mail.hzau.edu.cn (H. He).
http://dx.doi.org/10.1016/j.jff.2014.03.022
1756-4646/

enzyme, hen egg white is reported to possess many functions such as anti-inflammatory, antimicrobial, ACE-inhibitory and antidiabetic activities (Lee et al., 2009; Liu et al., 2010; You, Udenigwe, Aluko, & Wu, 2010; Yu et al., 2011). However, few reports have been published on the utilization of desalted duck egg white.

Our previous study has confirmed that the peptides prepared from desalted duck egg white (DPs) can bind calcium and prevent calcium from precipitating *in vitro* (Zhao, He, Hu, Hou, & Wang, 2013), but the effect of DPs *in vivo* is still unclear. The effects of peptides or corresponding peptides–calcium complexes such as soybean peptides and soybean peptides–calcium complexes on calcium absorption have been reported, but which is more effective *in vivo* is still unknown. It has been reported that the phosphorylation of soybean peptides resulted in considerable improvement in their calcium binding activities (Lee et al., 2005). We also found that DPs had a higher Ser content (7.10%) than soybean peptides (5.90%). Thus, we presume phosphorylated DPs (PDPs) can be more effective than DPs *in vivo*. The aim of the current study was to estimate the effects of DPs, PDPs and DPs–calcium complexes (DPs-Ca) on bone tissues and bone mechanical properties of rapidly growing rats.

2. Materials and methods

2.1. Materials

Salted duck egg white with a sodium chloride content of 7% and a protein content of 10% was donated by Hubei Shendan Healthy Food Co., Ltd. (Wuhan, China). CPPs with a protein content of 80% were purchased from Beijing Huiyuankang Science and Technology Co., Ltd. (Beijing, China). Protamex was the product from Novozymes (Copenhagen, Denmark). Calcium gluconate, $CaCO_3$, sodium trimetaphosphate (STMP) and the other reagents were of analytical grade.

2.2. Preparation of DPs, PDPs and DPs-Ca

Salted duck egg white was desalted by the electrodialysis equipment (QQX, Zhejiang Qianqiu Environmental Water Treatment Co., Ltd, Hangzhou, China) as reported in our previous work (Zhao et al., 2013). The desalted duck egg white (protein content 3%) was then denatured for 30 min in boiling water and cooled to 50 °C. The pH was adjusted to 6.5 prior to the addition of protamex (E:S = 1:25) and maintained at 6.5. After 3.5 h, the hydrolyzed solution was bathed in boiling water for 10 min to inactivate the enzyme. Finally, the mixture was centrifuged at 3000 × g for 10 min, and the supernatant was filtered through a hollow fiber membrane with a molecular weight cutoff of 5 kDa (PLCC, Millipore, Billerica, MA, USA). The filtrate was lyophilized and named as DPs. In our previous unpublished work, five amino acid sequences of DPs that may contribute to the ability of binding calcium were identified by HPLC-ESI/MS and they were Val-His-Ser-Ser (VHSS), Gln/Lys-Met-Lys (Q/KMK), Ile-Leu-Lys-Asn(ILKN), Phe-Glu-Lys-Asn (FEKN) and Phe-Gln-Thr (FQT). (For details, refer to the supplementary data in the online version at doi:10.1016/j.jff.2014.03.022.)

PDPs were prepared by dissolving DPs in distilled water (protein content 3%) at pH 9.0 and then were phosphorylated in the presence of STMP (0.2 g/g peptide). After stirring at 50 °C for 2 h, the pH of the mixture was adjusted to neutral and then centrifuged at 3000 × g for 10 min. Subsequently, the supernatant was nanofiltrated with a membrane (M_W cutoff 360 Da) (LNG-02–360, Shanghai Laungy Membrane Filtration Technology Co., Ltd, Shanghai, China), and the retentate with a molecular weight higher than 360 Da was collected and diluted with distilled water. This nanofiltration and dilution step was repeated three times to remove the redundant STMP. Finally, the retentate was collected as PDPs and lyophilized for future use.

DPs-Ca were prepared by mixing 4% DPs solution with $CaCl_2$ (0.4 g/g peptide) at pH 8.5, followed by 30 min incubation at 55 °C, and the addition of 95% ethanol to a final ethanol concentration of 70%. After centrifugation at 3000 × g for 5 min, the supernatant was discarded and the precipitation was collected and rinsed three times with 70% ethanol to remove the superfluous Ca^{2+}. Finally, the precipitation was freeze-dried and labeled as DPs-Ca.

2.3. Chemical analysis

Peptide contents of DPs, PDPs and DPs-Ca were determined by folin phenol reagent (Lowry, Rosebrough, Farr, & Randall, 1951), using bovine serum albumin as a standard. The phosphorus content of PDPs was determined by the standard for phosphorus determination in food established by P.R. China (GB/T 5009.87–2003). The calcium concentration of DPs-Ca was measured with an atomic absorption spectrophotometer (AA-6300C, Shimadzu, Kyoto, Japan).

2.4. Animal and diet

Four-week-old weanling Wistar male rats were obtained from Hubei Laboratory Animal Research Center and kept in stainless steel wire-bottomed cages under standard environmental conditions of 22 ± 2 °C, 60 ± 5% humidity and 12 h dark/light cycle. They were fed the AIN-93 diet and allowed free access to deionized water. The AIN-93 diet (low calcium diet) was purchased from Trophic Animal Feed High-tech Co., Ltd. (Nantong, China) and was slightly modified (low calcium diet containing 21.85% casein, 0.3% cystine, 48.1% maize starch, 10% sucrose, 10% maize oil, 5% cellulose, 3.5% mineral mix containing 0.1% calcium, 1% vitamin mixtures, and 0.25% choline bitartrate). After 1 week of acclimation, a total of 90 rats were randomly divided into 10 groups with nine rats in each group (Table 1).

The test substances were orally administered via a gavage tube of 1 mL/100 g bw and the low and high dosages were determined according to the calcium content. Based on the recommended daily human calcium intake of 800 mg Ca/60 kg bw (13.33 mg Ca/kg bw), the low and high dosages of calcium were designed as five and ten times the intake of human dosage, namely 66.67 mg Ca/kg bw and 133.3 mg Ca/kg bw, respectively. As DPs-Ca have a calcium content of 9.66% and a peptide content of 69.54%, the low and high dosages of peptides were determined to be 480 mg/kg bw (69.54% × 66.67/9.66%) and 960 mg/kg bw (69.54% × 133.33/9.66%), respectively. Given the peptide contents of 81.34% in CPPs, 82.97% in DPs, and 78.86% in PDPs, the low dosages of CPPs, DPs and PDPs were calculated to be about 600 mg/kg bw, and the high dosages of