Available at www.sciencedirect.com

SciVerse ScienceDirect

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

Fried egg digest decreases blood pressure in spontaneous hypertensive rats

Kaustav Majumder[a], *Sareh Panahi*[a,b], *Susan Kaufman*[b], *Jianping Wu*[a,*]

[a]*Department of Agricultural Food and Nutritional Science, Faculty of Agriculture Life and Environmental Sciences, University of Alberta, Edmonton, AB, Canada*

[b]*Department of Physiology, Cardiovascular Research Centre, Faculty of Medicine, University of AB, Edmonton, AB, Canada*

ARTICLE INFO

Article history:
Received 18 July 2012
Received in revised form
30 September 2012
Accepted 3 October 2012
Available online 23 November 2012

Keywords:
Fried whole egg digest
Spontaneously hypertensive rats
Angiotensin converting enzyme-I inhibition
Blood pressure
Circadian rhythm

ABSTRACT

Our previous study showed that *in vitro* simulated gastrointestinal digestion of fried whole egg (FWE) released several peptides with angiotensin converting enzyme-I (ACE-I) inhibitory properties. The present study evaluated *in vivo* blood pressure lowering effect of FWE digest in spontaneously hypertensive rats (SHR). Twelve to fourteen weeks old male SHRs were surgically implanted with telemetric blood pressure (BP) measuring devices. After one week recovery, animals were randomly allocated to three groups at dosage of FWE digest 0 (control), 100, and 1000 mg of FWE digest/kg body weight for 3 days ($n = 8$), and the BP was recorded continuously. The 1000 mg/kg BW group showed a significant decrease in BP and also rectified the impaired circadian blood pressure rhythm compared to the control group. This study indicates a potential blood pressure lowering effect of egg consumption.

1. Introduction

Egg is an economically and nutritionally important food commodity, consumed worldwide. It contains water (75%), proteins (12%), lipids (12%), vitamins, carbohydrates, and minerals (Cotterill & Geiger, 1977). Egg proteins are well known for their excellent digestibility and the highest nutritional quality among all food proteins, providing all the essential amino acids that closely match human requirements (Mann, 1988). Despite the fact that egg is an excellent source of well-balanced nutrients, there is a fear over egg intake due to controversial perception over the cholesterol in eggs. However, recent scientific evidence suggests that there is no direct relationship between egg intake and the incidence of cardiovascular disease (CVD) (Griffin, 2011; Howell, McNamara, Tosca, Smith, & Gaines, 1997) and serum cholesterol concentration (Song & Kerver, 2000). Cross-sectional and population based human clinical studies have shown that egg consumption made important nutritional contribution to the human nutrition (Song & Kerver, 2000).

Egg is also a rich source of various bioactive proteins and peptides that may exert benefits for human health (Aleixandre, Miguel, & Muguerza, 2008; Hartmann & Meisel, 2007; Korhonen & Pihlanto, 2003; Martinez-Maqueda, Miralles, Recio, & Hernandez-Ledesma, 2012). Antimicrobial, anticancer, antioxidative, antihypertensive or angiotensin converting enzyme-I (ACE-I) inhibitory peptides have been previously reported from eggs (Kovacs-Nolan, Phillips, & Mine, 2005); among them, antihypertensive or ACE-I inhibitory peptides are of special interest due to the prevalence of

* *Corresponding author.* Address: 4-10 Ag/For Centre, Edmonton, AB, Canada T6G 2P5. Tel.: +1 780 492 6885; fax: +1 780 492 4265.
E-mail address: jianping.wu@ualberta.ca (J. Wu).

Abbreviations: SHR, spontaneously hypertensive rats; FWE, fried whole egg; ACE-I, angiotensin converting enzyme-I; MAP, mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate.

1756-4646/$ - see front matter
http://dx.doi.org/10.1016/j.jff.2012.10.004

hypertension (Aleixandre et al., 2008; Miguel & Aleixandre, 2006). Hypertension or high blood pressure is identified as one of the major risk factors for CVD (Chockalingam, 2008). CVD is the major cause of morbidity and mortality, worldwide. According to World Health Organization (WHO), 17.3 million people died from CVDs in 2008 (World Health Organization, 2011).

ACE-I is a key enzyme for regulation of blood pressure through renin–angiotensin system (RAS). RAS plays a vital role in the regulation of blood pressure in mammals (Oparil & Haber, 1974; Zaman, Oparil, & Calhoun, 2002). Renin (EC 3.4.99.19) cleaves the N-terminal of angiotensinogen, and thus produces angiotensin-I (ANG-I) (Nicholls, 1985; Peach, 1977). ANG-I is then subsequently converted into a potent vasoconstrictor octapeptide, angiotensin-II (ANG-II) by ACE-I; simultaneously ACE-I also inactivates bradykinin, a vasodilator (Ondetti, Rubin, & Cushman, 1977). ACE-I is one of the target enzymes for antihypertensive drugs development and its principal physiological function in cardiovascular homeostasis has been well described (Kamper, 1991). Current pharmacological ACE-I inhibitors, such as captopril, benazepril, and enalapril, are the first-line therapy for hypertension (Brown & Vaughan, 1998; Lonn et al., 1994); however, these pharmacological inhibitors are often associated with unavoidable adverse side effects (Atkinson & Robertson, 1979). Therefore, there is an increasing interest in ACE-I inhibitory peptides derived from food proteins as an alternative therapy for the management of hypertension (Hong et al., 2008).

Our previous study showed that simulated gastrointestinal digestion of fried whole egg (FWE) could produce various ACE-I inhibitory peptides (Majumder & Wu, 2009), indicating the possibility of *in vivo* antihypertensive activity. Therefore, the objective of the present study was to evaluate antihypertensive activity of fried whole egg (FWE) digest upon oral administration in spontaneously hypertensive rats (SHR).

2. Materials and methods

2.1. Preparation of fried whole egg

Fresh white-shell, non-fertilized chicken eggs were obtained from Poultry Research center farm of the University of Alberta (Edmonton, AB, Canada). To make fried whole egg samples, whole egg was homogenized and placed in a frying pan (preheated to 350 °F) for 80 s, 40 s for each side, without oil. After cooling to room temperature, the fried egg samples were frozen immediately at −20 °C till stimulated *in vitro* gastrointestinal digestion.

2.2. Stimulated digestion of fried egg

Fried whole egg was digested under simulated gastrointestinal conditions, sequentially with pepsin (porcine gastric mucosa; Sigma–Aldrich, Oakville, ON, Canada) followed by pancreatin (porcine pancreas; Sigma–Aldrich, Oakville, ON, Canada). Fried egg was mixed with distilled water into 5% (w/v) slurry; after heating the slurry at 80 °C for 10 min, the temperature was adjusted to 37 °C by putting it into an ice bucket, and the pH was adjusted to 2 by adding 1 M HCl. The slurry was first digested by pepsin (4%, w/w, enzyme/egg protein) for 3 h and then the pH was increased to 7.5 to inactivate the enzyme by adding 1 M NaOH solution; the slurry was then subjected to further pancreatin (2%, w/w) digestion for another 3 h. The hydrolysis was terminated by raising the temperature to 95 °C and maintaining it for 10 min; the digests were then freeze-dried without further centrifugation separation. The digestion was carried out through Titrando (Metrohm, Herisan, Switzerland) for maintaining constant pH during the course of the hydrolysis. The temperature of the sample during digestion was maintained with a circulating water bath.

2.3. Animal model and environmental condition

The experimental procedures were approved by the University of Alberta Animal Welfare Committee in accordance with the guidelines issued by the Canada Council on Animal Care. 12–14 weeks (275–300 g) old male spontaneous hypertensive rats (SHR) were obtained from Charles Rivers Canada. They were kept in the University of Alberta animal facility for a week for acclimatization, exposed to 12 h cycle of light (light on from 6 am to 6 pm) in a humidity and temperature-controlled (@ 23 °C) environment. All rats were fed with standard rat chow (0.3% NaCl) and water *ad libitum*.

2.4. Anesthesia and surgical procedure

Anesthesia before the surgery was induced with Isoflurane (1.5%). Body temperature was maintained at 37 °C (Homethermic Blanket, Harvard Apparatus, Saint-Laurent, QC, Canada) during the surgery. Animals were chronically instrumented with DSI telemetry probes (PA-C40; Data Sciences International, Minneapolis, MN) according to the manufacture's manual for continuous monitoring of mean arterial pressure (MAP) and heart rate (HR) without any physical restriction. The left femoral artery was exposed by an approximately 2 cm long incision on the rat's left groin area. The flexible tip of the telemetry probe was then inserted into the femoral artery and advanced up to the abdominal aorta. The catheter was then secured at the point of entry to the vessel by using a 4/0 silk suture. The transmitter was inserted under the skin, on the back just to the left of the spine and secured with 4/0 silk suture. After surgery, the rats were caged individually and allowed for one week for recovery and also helped to acclimatize with the recording room. During the week of recovery, animals were fed with 50 mL of Ensure (Abbott Nutrition, Saint-Laurent, QC, Canada), rodent chow and water *ad libitum*. To reduce the pain the rats received one dose (0.03 mg/kg BW) of buprenorphine (0.3 mg/mL) (Animal Resources centere, McGill University, Montreal, QC, Canada), just after the surgery and then continued for next 2–3 days based on the condition of the individual animal.

2.5. Data acquisition and signal processing

Recording was made in a quiet room with minimal electrical interference. Prior to installation, calibration of every telemetry device was verified. Throughout the blood pressure (BP) recording an atmospheric-pressure monitor (Model APR-1)