In ovo ghrelin affects antioxidant enzymes (SOD and GPx) in newly-hatched chicken

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Abstract

Ghrelin is a regulatory peptide with specific anti-oxidative effects. The aim of this study was to investigate the activity of two antioxidant enzymes includes superoxide dismutase (SOD) and glutathione peroxidase (GPx) in erythrocyte of newly-hatched chicken following *in ovo* administration of ghrelin. The fertilized eggs were divided into four experimental groups; control G1 (without injection), group G2 (*in ovo* injected with 50 ng/egg ghrelin), group G3 (*in ovo* injected with 100 ng/egg ghrelin), group G4 (*in ovo* injected with 150 ng/egg ghrelin). At d 10 of incubation, *in ovo* injections of ghrelin were conducted. SOD and GPx activity in erythrocytes were determined by laboratory analysis of whole blood taken from newly-hatched chicks. There were no significant changes in SOD activity following *in ovo* administration of different concentrations of exogenous ghrelin. But, in groups G3 and G4 (100 ng and 150 ng ghrelin/ egg), significant elevation in activity of GPx has been observed ($P< 0.05$). It was concluded that *in ovo* administrated ghrelin can increase antioxidant defense by increase in GPx activity in newly-hatched chickens.

Key words: ghrelin; antioxidant enzymes; oxidative damage; *in ovo* injection.

Introduction

Ghrelin is a multifunctional regulatory peptide with anti-oxidative effect (Tong *et al.*, 2012; Kawczynska-Drozdz *et al.*, 2006; Obay *et al.*, 2008; Çetin *et al.*, 2011a). Following identification of ghrelin in mammals by Kojima et al., (1999), so many relative studies were published on ghrelin properties and its physiological importance. Studies conducted during last decade suggests various specifies and functions for ghrelin, such as GH-releasing (Hashizume *et al*., 2005), hematopoiesis (Akarsu *et al*., 2007; Lotfi *et al*., 2011a), food intake and energy balance and regulation (Nakazato et al., 2001), endocrine/paracrine roles in pancreas (Lotfi *et al*., 2011b; Lotfi et al., 2011c; Stevanović *et al*., 2007; Dezaki et al., 2008), and the anti-oxidative effects (Tong *et al.*, 2012; Kawczynska-Drozdz *et al.*, 2006; Obay *et al.*, 2008; Çetin *et al.*, 2011a). The chicken ghrelin has 26 amino acids with 54% homology to rat ghrelin (Fig. 1; Saito et al., 2002; Kaiya *et al*., 2008).

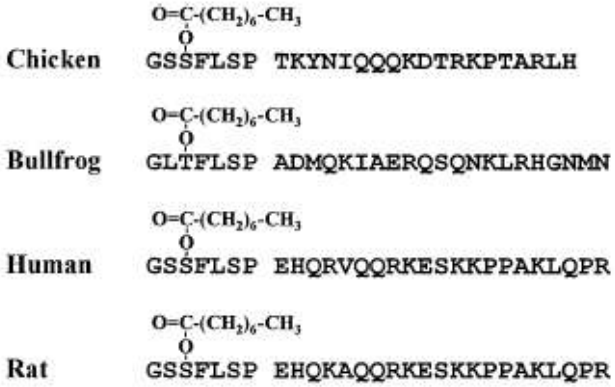

Fig 1. Amino acid sequence of ghrelin peptide in various animal species (Saito et al., 2002).

The maternal ghrelin has been identified in albumen and yolk of fertilized chicken egg (Yoshimura *et al*., 2009). So, chicken ghrelin may have numerous functional effects in chicken embryo development, similar with mammalian ghrelin. In this regard, our *in ovo* studies show considerable regulatory effects of ghrelin on some of endocrine hormones (includes and prolactin insulin) (Lotfi *et al*., 2011a; 2011b; 2011c), hematopoiesis (Lotfi *et al*., 2011a), and intestinal development (Lotfi *et al*., 2012) in chicken.

The chicken embryo has challenges with oxidative stress, and during the final 2 weeks of embryonic development, notable changes in expression of superoxide dismutase (SOD) and glutathione peroxidase (GP_X) were occurred (Wilson et al., 1992). Investigations of Kheradmand *et al*., (2009; 2011) had mentioned specific anti-oxidant effect for rat ghrelin. But this effect was not studied in chicken or for chicken ghrelin.

So, the aim of this study was to investigation on activity of two antioxidant enzymes (SOD and GPx) in erythrocyte of newly-hatched chicken followig *in ovo* administration of ghrelin.

Materials and methods

In present study, 200 fertilized eggs were obtained from broiler breeder flock (Ross 308). The eggs were divided into four experimental groups; control G1 (without injection), group G2 (*in ovo* injected with 50 ng/egg ghrelin), group G3 (*in ovo* injected with 100 ng/egg ghrelin), group G4 (*in ovo* injected with 150 ng/egg ghrelin). All of eggs were incubated with normal incubation condition (37.8 $^{\circ}$C and 60%: RH). Exogenous ghrelin (Rat Ghrelin – Sigma-Aldrich, USA), dissolved in 1% acetic acid solvent (according to company brochure) and required concentrations of ghrelin were prepared. At d 10 of incubation, *in ovo* injection was conducted for G2, G3 and G4. Before injection, egg shells were marked with waterproof marker for identification of air cell position and optimum site for *in albumen* injection with 22G needles. The *in ovo* injection was done in hygiene room with 37 $^{\circ}$C for avoiding any thermal stress for embryo. After hatching, the blood of newly-hatched chickens was collected in EDTA coated tubes. The activities of SOD (EC: 1.15.1.1) and GP_X (EC: 1.11.1.9) were measured by Alyson auto-analyzer and commercial kits. Enzymatic activities were expressed as U/l. Obtained data were analyzed with SAS software (Ver. 9.1) and the differences between means were detected via Duncan multiple range test ($P<0.05$).

Results and discussion

According to table1, there were no any significant changes for SOD activity following *in ovo* administration of different concentrations of exogenous ghrelin. But there was drastic elevation in GPx activity following ghrelin administration, so in groups G3 and G4 (100 ng and 150 ng ghrelin/ egg), significant elevation in activity

of GPx has been occurred (P< 0.05), whereas difference between G2 (50 ng ghrelin/ egg) and G1 (control or intact group) for GPx was not significant.

Table. 1. Activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in erythrocyte of newly-hatched chicken following *in ovo* ghrelin administration

Experimental groups	Injected dosage (ng/egg)	SOD (U/L)	GPx (U/L)
G1 (intact)	-	0.93	169.03[b]
G2	50	1.03	175.70 [ab]
G3	100	0.80	181.07 [a]
G4	150	0.73	177.33 [a]
P value	-	0.4471	0.0399
SEM[1]	-	0.3203	2.3756

different letters (a or b) shows significant difference; [1] SEM, based on pooled estimate of variance.

Hatching time is considered to be a period of high oxidative stress due to long-chain poly unsaturated fatty acids accretion in tissues (Cherian *et al*., 1997; Schaal, 2008). The pulmonary respiration and subsequent increase in rate of oxidative metabolism and the hatchlings process are expected to react with a compensatory induction of endogenous antioxidants (Speake *et al*., 1998). For the purpose of elimination of oxidative damages for embryo and at hatching process, *in ovo* administration of antioxidant compounds such as sodium selenite and vitamin C (Wang *et al*., 2008) had markedly abated oxidative damages in chick embryo or newly-hatched chicks.

It was suggested that ghrelin has free radical scavenging capacity (Çetin *et al*., 2011b). In a previous study (Kheradmand *et al*., 2009), it was reported that GPx activity in rat testis was increased and SOD activity was unchanged by administration of 1 nmol kg^{-1} ghrelin for 10 d. They had suggested that greater concentrations of ghrelin may be able to activate SOD enzyme. In theirs other experiment (Kheradmand *et al*., 2011) conducted on rat ovary, it reported that SOD is major antioxidant enzyme affected by administrated ghrelin, and GPx is not affected significantly. Çetin *et al*., (2011a, 2011b) had stated that ghrelin pretreatment enhances antioxidant defense via activation of antioxidant enzymes, against oxidative injury. In present study (table1), according to Kheradmand *et al*., (2009) findings, *in ovo* administration of ghrelin caused increased rate of GPx in comparison with control (G1). Also present finding is in agreement with Barazzoni *et al*., (2011) that reported on elevation in GPx activity of rat muscle due to injection of Acyl-ghrelin. In present study *in ovo* administration of ghrelin (100 ng or 150 ng/ egg) cause increased GPx activity, but there was not significant change for SOD (table 1). It seems that ghrelin has antioxidant effect due to activation of both or one of major antioxidant enzymes (SOD and GPx). In chicken, *in ovo* or maternal ghrelin may has antioxidant role, and make antioxidant defens in post-hatch and neonatal life. It was concluded that *in ovo* administrated ghrelin can increase antioxidant defense by increase in GPx activity in newly-hatched chickens.

References

1) Akarsu S, Ustundag B, Gurgoze MK, Sen Y and Aygun AD (2007). Plasma ghrelin levels in various stages of development of iron deficiency anemia. *Journal of Pediatric Hematology/ Oncology*. **29**: 384-387.

2) Barazzoni R, Zanetti M, Semolic A, Cattin MR, Pirulli A, Cattin L, Guarnieri G. (2011). High-fat diet with acyl-ghrelin treatment leads to weight gain with low inflammation, high oxidative capacity and normal triglycerides in rat muscle. *PLoS One*, **6**: e26224.

3) Çetin E, Kanbur M, Çetin N, Eraslan G, Atasever A. (2011a). Hepatoprotective effect of ghrelin on carbon tetrachloride-induced acute liver injury in rats. *Regulatory Peptides*, **171**: 1-5.

4) Çetin N, Boyraz U, Çetin E. (2011b). Ghrelin alleviates tilmicosin-induced myocardial oxidative stress in rats. *Journal of Animal and Veterinary Advances*, **10**: 2038- 2042.

5) Cherian G, Gopalakrishnan N, Akiba Y, Sim JS, (1997). Effects of maternal dietary 18:3 n-3 acids on the accretion of long chain polyunsaturated fatty acids in the tissue of developing chick embryo. *Biology of the Neonate*, **72**: 165–174.

6) Dezaki K, Sone H, Yada T, (2008). Ghrelin is a physiological regulator of insulin release in pancreatic islets and glucose homeostasis. *Pharmacology & Therapeutics*, **118**: 239–249.

7) Hashizume T, Horiuchia M, Nonakaa S, Kasuyab E, Kojimac M, Hosodad H, Kangawa K, (2005). Effects of ghrelin on growth hormone secretion in vivo in ruminants. *Regulatory Peptides*, **126**: 61–65.

8) Kaiya H, Miyazato M, Kangawa K, Peter RE, Unniappan S, (2008). Ghrelin: a multifunctional hormone in non-mammalian vertebrates. *Comparative Biochemistry and Physiology, Part A*, **149**: 109–128.
9) Kawczynska-Drozdz A, Olszanecki R, Jawien J, Brzozowski T, Pawlik WW, Korbut R, Guzik TJ, (2006). Ghrelin inhibits vascular superoxide production in spontaneously hypertensive rats. *American Journal of Hypertension*, **19**: 764-767.
10) Kheradmand A, Alirezaei M, Asadian P, Rafiei Alavi E, Joorabi S, (2009). Antioxidant enzyme activity and MDA level in the rat testis following chronic administration of ghrelin. *Andrologia*, **41**: 335–340.
11) Kheradmand A, Alirezaei M, Birjandi M, (2011). Ghrelin promotes antioxidant enzyme activity and reduces lipid peroxidation in the rat ovary. *Regulatory Peptides*, **162**: 84–89.
12) Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K, (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*, **402**: 656–660.
13) Lotfi A, Shahryar HA, Narimani-Rad M, Sharaf JD, (2011a). Changes in the differential leukocyte count in newly hatched chicks following in ovo ghrelin administration. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, **17**: 949-952.
14) Lotfi A, Aghdam-Shahryar H, Ghiasi- Ghalehkandi J, Kaiya H, Maheri-Sis N, (2011b). Effect of in ovo ghrelin administration on subsequent serum insulin and glucose levels in newly-hatched chicks. *Czech Journal of Animal Science*, **56**: 377–380.
15) Lotfi A, Aghdam Shahryar AH, Ghiasi Ghale-kandi J, Kaiya H, Ahmadzadeh A, (2011c). In ovo Administration of Ghrelin and Subsequent Prolactin level in Newly-hatched Chicks. *Journal of Poultry Science*, **48**: 135- 137.
16) Lotfi A, Aghdam Shahryar H, Narimani-Rad M, Rasoolian H, (2012). Effect of in ovo ghrelin administration on internal organ weights in newly-hatched chickens. In proceeding of *International Animal Science Congress of Turkish and Relative Communicates*, 21- 24 Sept 2012, Suleyman Demirel University, Isparta, Turkey.
17) Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, Matsukura S, 2001. A role for ghrelin in the central regulation of feeding. *Nature*, 409: 194-198.
18) Obay BD, Taşdemir E, Tümer C, Bilgin HM, Atmaca M, 2008. Dose dependent effects of ghrelin on pentylenetetrazole-induced oxidative stress in a Rat seizure model. *Peptides*, 29: 448-455.
19) Saito ES, Kaiya H, Takagi T, Yamasaki I, Denbow DM, Kangawa K, Furuse M. (2002). Chicken ghrelin and growth hormone-releasing peptide-2 inhibit food intake of neonatal chicks. *European Journal of Pharmacology*, **453**:75-79.
20) Schaal TP, (2008). The Effects of in ovo feeding of fatty acids and antioxidants on broiler chicken hatchability and chick tissue lipids. Thesis for degree of Honors Baccalaureate of Science in Animal Science, Oregon state University.
21) Speake BK, Murray AMB, Noble RC, (1998). Transport and transformation of yolk lipids during development of the avian embryo. *Progress in Lipid Research*, **37**: 1–32.
22) Stevanović D, Milošević V, Starčević VP, Severs WB, (2007). The effect of centrally administered ghrelin on pituitary ACTH cells and circulating ACTH and corticosterone in rats. *Life Science*, **80**: 867–872.
23) Tong XX, Wu D, Wang X, Chen HL, Chen JX, Wang XX, Wang XL, Gan L, Guo ZY, Shi GX, Zhang YZ, Jiang W, (2012). Ghrelin protects against cobalt chloride-induced hypoxic injury in cardiac h9c2 cells by inhibiting oxidative stress and inducing autophagy. *Peptides*, **38**: 217-27.
24) Wang YM, Wang HJ, Peng SQ, (2008). Lipid peroxidation and antioxidant defense impairment in the hearts of chick embryos induced by in ovo exposure to Fusarium mycotoxin butenolide. *Toxicon*, **52**: 781-786.
25) Wilson JX, Lui EMK, DEL Maestro RF, (1992). Developmental profiles of antioxidant enzymes and trace metals in chick embryo. *Mechanisms of Ageing and Development*, **65**: 51-64.
26) Yoshimura Y, Tsuyuki C, Subedi K, Kaiya H, Sugino T, Naoki I, (2009). Identification of ghrelin in fertilized eggs of chicken. *Journal of Poultry Science*, **46**: 257-259.