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Original Contribution

Selenoproteins protect against avian nutritional muscular dystrophy by metabolizing peroxides and regulating redox/apoptotic signaling

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ABSTRACT

Nutritional muscular dystrophy (NMD) of chicks is induced by dietary selenium (Se)/vitamin E (Vit. E) deficiencies and may be associated with oxidative cell damage. To reveal the underlying mechanisms related to the presumed oxidative cell damage, we fed four groups of 1-day-old broiler chicks ($n = 40$/group) with a basal diet (BD; 10 μg Se/kg; no Vit. E added, −Se −Vit. E) or the BD plus all-rac-α-tocopheryl acetate at 50 mg/kg (−Se +Vit. E), Se (as sodium selenite) at 0.3 mg/kg (+Se −Vit. E), or both of these nutrients (+Se +Vit. E) for 6 weeks. High incidences of NMD (93%) and mortality (36%) of the chicks were induced by the BD, starting at week 3. Dietary Se deficiency alone also induced muscle fiber rupture and coagulation necrosis in the pectoral muscle of chicks at week 3 and thereafter, with increased ($P < 0.05$) malondialdehyde, decreased ($P < 0.05$) total antioxidant capacity, and diminished ($P < 0.05$) glutathione peroxidase activities in the muscle. To link these oxidative damages of the muscle cells to the Se-deficiency-induced NMD, we first determined gene expression of the potential 26 selenoproteins in the muscle of the chicks at week 2 before the onset of symptoms. Compared with the +Se chicks, the −Se chicks had lower ($P < 0.05$) muscle mRNA levels of *Gpx1, Gpx3, Gpx4, Sepp1, Selo, Selk, Selu, Selh, Selm, Sepw1,* and *Sep15*. The −Se chicks also had decreased ($P < 0.05$) production of 6 selenoproteins (long-form selenoprotein P (SelP-L), GPx1, GPx4, Sep15, SelW, and SelN), but increased levels ($P < 0.05$) of the short-form selenoprotein P in muscle at weeks 2 and 4. Dietary Se deficiency elevated ($P < 0.05$) muscle p53, cleaved caspase 3, cleaved caspase 9, cyclooxygenase 2 (COX2), focal adhesion kinase (FAK), phosphatidylinositol 3-kinase (PI3K), phospho-Akt, nuclear factor-κB (NF-κB), p38 mitogen-activated protein kinase (p38 MAPK), phospho-p38 MAPK, phospho-JNK, and phospho-ERK and decreased ($P < 0.05$) muscle procaspase 3, procaspase 9, and NF-κB inhibitor α. In conclusion, the downregulation of SelP-L, GPx1, GPx4, Sep15, SelW, and SelN by dietary Se deficiency might account for induced oxidative stress and the subsequent peroxidative damage of chick muscle cells via the activation of the p53/caspase 9/caspase 3, COX2/FAK/PI3K/Akt/NF-κB, and p38 MAPK/JNK/ERK signaling pathways. Metabolism of peroxides and redox regulation are likely to be the mechanisms whereby these selenoproteins prevented the onset of NMD in chicks.

Nutritional muscular dystrophy (NMD)[1] [1,2] was one of the first characterized selenium (Se)/vitamin E (Vit. E) deficiency diseases in chicks and other food-producing species. It is likely that Se is the primary nutritive factor [3–5], whereas Vit. E is only partially effective, or not at all [4,6,7], in preventing this disease in poults. Numerous attempts [1,2,4–6,8] have been made to elucidate the pathogenesis of NMD over the past 70 years. Although the hypothesis of increasing oxidant generation and consequent oxidative damage has been proposed as the most likely explanation, specific molecular mechanisms underlying this classical disease induced by antioxidant nutrient deficiencies remain unclear.

To tackle this problem, we have previously determined expression profiles of 14 selenoprotein genes (*Gpx1, Gpx4, Sepp1, Selo,*

Abbreviations: ActB, β-actin; Akt, protein kinase B; COX2, cyclooxygenase 2; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; GPx, glutathione peroxidase; IκBα, NF-κB inhibitor α; JNK, c-Jun N-terminal kinase; MDA, malondialdehyde; NF-κB, nuclear factor-κB; NMD, nutritional muscular dystrophy; p38 MAPK, p38 mitogen-activated protein kinase; p53, tumor suppressor p53; PI3K, phosphatidylinositol 3-kinase; Q-PCR, real-time quantitative polymerase chain reaction; ROS, reactive oxygen species; Sec, selenocysteine; Sel, selenoprotein; Sep15, selenoprotein 15; SOD, superoxide dismutase; TUNEL, TdT-mediated dUTP nick-end labeling; Vit. E, vitamin E

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Selk, *Sepw1*, *Sepn1*, *Sep15*, *Sels*, *Selt*, *Sepx1, Seli, Txnrd1,* and *Txnrd2*) in muscle and liver of chicks fed Se-deficient (0.01 mg/kg) or -adequate (0.3 mg/kg) diets for 6 weeks [9]. Dietary Se deficiency decreased ($P < 0.05$) mRNA levels of 7 selenoprotein genes (*Gpx1, Gpx4, Sepp1, Selo, Selk, Sepw1,* and *Sepn1*) in those tissues. However, the 6-week time point was too far from the initial pathogenesis of NMD in chicks to associate these molecular changes with the onset of the disease, which occurs within 3 weeks of consuming a low-Se diet. Because 26 selenoprotein genes have been discovered in the avian genome [10], it is necessary to reveal how the expression of all these genes in chick muscle responds to dietary Se and Vit. E deficiencies at earlier time points, before the onset of NMD. Whereas the mRNA levels of two abundant selenoproteins (glutathione peroxidase 1 (GPx1) and GPx4) are highly regulated by Se status in poults [9,11], the production of the pertinent proteins and the respective gene expression are not always consistent. In fact, the protein levels are more relevant to physiological functions or metabolic phenotypes. Up to now, at least 8 selenoproteins, including GPx1, GPx3, GPx4, selenoprotein 15 (Sep15), selenoprotein P (SelP), selenoprotein X (SelX), selenoprotein W (SelW), and selenoprotein N (SelN), have been reported to function as major peroxide scavengers or to be involved in muscle integrity and function [9,12–17]. Thus, the protein production of these key selenoproteins should be determined, along with their respective mRNA levels, in muscle before and after the onset of NMD.

In relation to NMD, the apoptosis of chicken skeletal muscle cells is regulated by p53-dependent and -independent pathways [18]. After p53 activates target caspases through mitochondrial cytochrome *c* release, the activation initiates pathways that lead to DNA fragmentation and chromatin condensation, hallmarks of apoptosis. Activation of the initiator caspases (such as caspases 8 and 9) and downstream caspases (such as caspases 3 and 7) mediates the progression of apoptosis in numerous cell types [19,20]. In addition, the muscle-specific activation of nuclear factor-κB (NF-κB) is elevated in dystrophin-deficient *Mdx* mice compared with normal mice [21]. The NF-κB activation is elevated in *Gpx1*$^{-/-}$ embryonic fibroblasts treated with hydrogen peroxide [22]. Levels of GPx1 activity and that of other selenoproteins may also be involved in this oxidation-mediated signaling, as Se-deficient macrophages have increased NF-κB activation and subsequent upregulation of cyclooxygenase 2 (COX2) [23]. Focal adhesion kinase (FAK) is able to activate several signal pathways including cell survival through the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) pathway in muscle cells [24]. Mitogen-activated protein kinases (MAPKs), including p38 MAPK, c-Jun N-terminal kinase (JNK), and extracellular signal-regulated kinase (ERK), are important in transducing extracellular stimuli into intracellular events [25,26]. The p38 MAPK and JNK pathways have essential roles in reactive oxygen species (ROS)-induced apoptosis [27,28]. Overexpression of GPx1 in mouse embryonic fibroblasts prevents the p38 MAPK phosphorylation at Thr-180/Tyr-182 under hypoxia that presumably induces oxidative stress [29]. However, little is known about how these protein kinases are associated with the avian NMD.

Therefore, we conducted this experiment to: (1) determine the effects of dietary Se and Vit. E deficiency on redox status and mRNA expression profiles of 26 selenoproteins in chick muscle before the onset of NMD, (2) explore how the muscle abundances of eight key selenoproteins involved in peroxide scavenging and muscle function are related to Se-deficiency-induced oxidative stress and NMD, and (3) link responses of the three redox/apoptotic signaling pathways (p53/caspase 9/caspase 3, COX2/FAK/PI3K/Akt/NF-κB, and p38 MAPK/JNK/ERK) in the muscle to the changes in selenoprotein expression associated with NMD.

Materials and methods

Animal, diet, and experimental design

The protocol was approved by the China Agricultural University Animal Care and Use Committee. Briefly, 1-day-old male broiler poults (total = 160) were purchased from Dafa Zhengda Poultry Co., Ltd. (Beijing, China) and randomly allocated to four dietary treatment groups ($n = 40$). The basal diet (Table 1) was composed of corn and soybean meal produced in the Se-deficient area of Sichuan, China, and was not supplemented with Se or Vit. E (−Se −Vit. E). The other three experimental diets were supplemented with rac-α-tocopheryl acetate (PureOne Biotechnology, Shanghai, China) at 50 mg/kg (−Se +Vit. E), Se (as sodium selenite, Sigma–Aldrich, St. Louis, MO, USA) at 0.3 mg/kg (+Se −Vit. E), or both of these nutrients (+Se +Vit. E). Analysis of these diets gave Se concentrations of 10, 14, 330, and 346 μg/kg, respectively. Chicks were housed in battery brooder cages with raised wire floors, and the room temperature was maintained at 30, 28, and 25 °C for the first, second, and subsequent weeks. Animals were provided free access to the designated diets in plastic troughs and deionized water in stainless steel troughs. The experiment lasted for 6 weeks. Individual body weights and cage feed intakes were measured weekly. General health, clinical symptoms of Se-deficiency diseases, and mortality were recorded daily. NMD was readily diagnosed by the appearance of grossly visible white striations running in the direction of the muscle fibers on the breast of the chicks (Supplementary Fig. 1A) [1].

Muscle sample collection and preparation

At weeks 2, 3, and 4 of the study, chicks ($n = 7$/group) were killed by decapitation, and then pectoral muscle samples were collected. The remaining animals were sampled at week 6. Muscle

Table 1
Composition of basal diet (as fed).

Ingredients	Content (g/kg)
Corn	789.0
Roasted soybean	150.0
$CaCO_3$	10.0
$CaHPO_4$	21.0
Salt	3.0
Choline	2.0
Trace mineral premix[a]	5.0
Vitamin premix[b]	0.5
Amino acid premix[c]	19.5
Total	1000.0
Nutrient composition (calculated)	
Metabolic energy (MJ/kg)	12.2
Crude protein (%)	19.8
Lysine (%)	0.8
Methionine (%)	0.5
Methionine + cysteine (%)	0.7
Calcium (%)	1.0
Available phosphorus (%)	0.5

The analyzed Se concentration in the basal diet was 10 μg/kg.

[a] Trace mineral premix provided/kg diet: $FeSO_4 \cdot 7H_2O$, 379 mg; $CuSO_4 \cdot 5H_2O$, 31.3 mg; $ZnSO_4 \cdot 7H_2O$, 177 mg; $MnSO_4 \cdot 5H_2O$, 154 mg; KI, 0.5 mg; and colistin sulfate, 40 mg.

[b] Vitamin premix provided/kg diet: retinyl acetate, 1500 IU; cholecalciferol, 200 IU; menadione, 5 mg; thiamin, 1.8 mg; riboflavin, 3.6 mg; calcium pantothenate, 10 mg; niacin, 35 mg; pyridoxol, 3.5 mg; *d*-biotin, 0.15 mg; and folacin, 0.55 mg (without rac-α-tocopheryl acetate).

[c] Amino acid premix provided/kg diet: L-lysine, 4630 mg; DL-methionine, 3160 mg; L-threonine, 2010 mg; L-tryptophan, 356 mg; isoleucine, 2020 mg; valine, 1610 mg; phenylalanine, 2690 mg; arginine, 2570 mg; and glycine, 900 mg.