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Effect Of Zinc Deficiency And Supplementation On Insulin Signaling In Chickens

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Ali Alkaladi: Effect Of Zinc Deficiency And Supplementation On Insulin Signaling In Chickens

ABSTRACT

The aim of this study to investigate the effect of either zinc (Zn) deficiency or supplementation on insulin synthesis and muscular insulin signals in chickens. A total of 90 one-day-old Hubbard male broiler were divided in to three groups ; control group (GI), Zn deficiency group (GII) and Zn supplemented group (GIII). After 21 days, blood , pancreas , liver and thigh muscle samples were taken to investigate blood glucose, liver glycogen, serum insulin, pancreatic cytosolic Zn, insulin receptor (IR), insulin receptor phosphorilation (IRP), insulin receptor substrate-1 (IRS-1), serine/thereonine kinase (AKT), phosphoinositide-3-kinase (PI3K) and glucose transporter protein 4(GLUT4) concentrations, IR and IRS-1 gene expressions. The results indicated that, Zn deficiency leads to decrease of hepatic glycogen, serum insulin , pancreatic cytosolic Zn, IRP, AKT, PI3K and GLUT4 concentrations and increase of blood glucose, while Zn supplementation reveres the result. So it can be concluded that Zn deficiency adversely affect insulin synthesis and muscular insulin signals, while Zn supplementation enforce both insulin synthesis and insulin signals in chickens.

Key wrods:

Introduction

Zinc is an essential trace element crucial for the function of more than 300 enzymes and it is important for cellular processes like cell division and apoptosis. Hence, the disturbances of zinc homeostasis have been associated with several diseases including diabetes mellitus, a disease characterized by high blood glucose concentrations as a consequence of decreased secretion or action of insulin. Zinc supplementation of animals and humans has been shown to ameliorate glycemic control in type 1 and 2 diabetes, the two major forms of diabetes mellitus, but the underlying molecular mechanisms have only slowly been elucidated. Zinc seems to exert insulin-like effects by supporting the signal transduction of insulin and by reducing the production of cytokines, which lead to beta-cell death during the inflammatory process in the pancreas in the course of the disease. Furthermore, zinc might play a role in the development of diabetes, since genetic polymorphisms in the gene of zinc transporter 8 and in metallothionein (MT)-encoding genes could be demonstrated to be associated with type 2 diabetes mellitus [11].

The total $Zn2^{+}$ content of the mammalian pancreas is high, and chiefly localized to the islet β-cell. It plays an important role in both insulin synthesis and storage. Indeed it's concentrations reach millimolar levels in the interior of the dense-core granule, where two $Zn2^{+}$ ions coordinate six insulin monomers to form the hexameric structure on which insulin crystals are based [3].

Zinc plays a crucial role in many cell functions; as a result, both zinc deficiency and excess of free zinc are toxic to mammalian cells. The abundance of zinc per cell is tissue dependent and the zinc content of pancreatic beta cells is among the highest in the body. In beta cells, zinc was proposed to be required for multiple steps in insulin synthesis and release, but conclusive evidence is lacking. After synthesis in the ER, pro-insulin is transported into the Golgi apparatus where immature, pale secretary ''progranules'' are formed . These granules contain pro-insulin-zinc hexamers which are further processed into mature insulin and C-peptide by the prohormone convertases PC1/3 and PC2 . After maturation, the zinc-insulin hexamers form water-insoluble crystals. It has been suggested that crystal formation increases the degree of conversion of soluble pro-insulin to insoluble insulin, but nearly normal pro-insulin processing occurs in patients with mutated histidine-B10 insulin, which cannot crystallize [6]. There are many studies on the role of zinc in insulin synthesis, storage and glucose homeostasis in mammals but this role in chicken is unknown , so this study was designed to monitor the effect of zinc deficiency and supplementation on

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insulin concentration, synthesis and mechanism of action on a molecular and cellular levels in chickens.

Material and Methods

Birds, Diets, and Treatments:

A total of 90 one-day-old male chicks were used in the 21-d experiment. Birds were randomly divided into three group; Control group, kept on the basal diet supplemented with 20 mg /kg added Zn from ZnSO4.7H2O to be contain (48.37 mg/Kg) Zn (NRC, 1994). Zn deficient group, kept on basal diet that contain 28,37 mg/kg Zn and Zn supplemented group, kept on basal diet supplemented with 60 mg/kg added Zn from ZnSO4.7H2O to be contain (88.37 mg/Kg) Zn. (Table I). The basal cornsoybean meal diet was formulated to meet or exceed the requirements for starter broilers (NRC, 1994) except for Zn and contained 28.37 mg of Zn/kg of diet on an as-fed basis, by analysis [7]. Chicks were maintained on a 24-h constant light schedule and allowed adlibitum access to experimental diets and tap water, which contained no detectable Zn.

Table 1: Composition of the basal diet for 1- to 21-day-old broilers(A)

Ingredient	Percentage	Calculated composition	
Corn	55.97	ME (Kcal/Kg)	2993
Soybean meal	36.00	CP[e] (%)	21.56
Soybean oil	3.60	Lys (%)	1.19
$CaHPO_4 H_2O$ [b]	1.95	Met (%)	0.54
$CaCO_3$ [b]	1.16	Met + Cys (%)	0.91
NaCL [b]	0.30	Ca[e] (%)	1.10
Met	0.20	Nonphytatephosphate	0.46
Micronutrient [c]	0.32	Zn [e]	28.37
Cornstarch + Zinc [d]	0.50		

(A) ingredient and nutrient composition reported on an as-fed basis

(b) reagent-grade

(c) provided per kilogram of diet: vitamin A (as all-*trans* retinol acetate), 15,000 IU; cholecalciferol, 3 900 IU; vitamin E (as all-*rac*-α-tocopherol acetate), 30 IU; vitamin K (as menadione sodium bisulfate), 3.0 mg; thiamin (as thiamin mononitrate), 2.4 mg; riboflavin, 9.0 mg; vitamin B6, 4.5 mg; vitamin B12, 0.021 mg; calcium pantothenate, 30 mg; niacin,

45 mg; folic acid, 1.2 mg; biotin, 0.18 mg; choline (as choline chloride), 700 mg; Cu, 8 mg; Mn, 100 mg; Fe, 80 mg; I, 0.35 mg; Se, 0.15 mg

(d) zinc supplement added in place of equivalent weight of cornstarch

(e) determined by analysis; each value based on triplicate determinations

Sample Collections and Analysis:

Blood samples were taken from each bird via cardiac puncture and then centrifuged to harvest serum for determination of insulin and glucose concentrations. Chicks were immediately killed by cervical dislocation. Pancreas and thigh muscle sample was frozen in liquid nitrogen until be used for laboratory investigation.

Assays:

Plasma glucose was quantitated by glucose oxidase-peroxidase method using the kit supplied by SPINREACT, Spain (Ref: 1001190). Serum insulin was determined using Ultra Sensitive Chickens Insulin ELISA Kit (Cat.No. E-EL-ch 1528, Elabscience, Beijin) following manufacturer instructions, liver Glycogen content was determined according to Caruso *et al,* [1] Zinc concentrations in pancreatic cell cytoplasm was determined by inductively coupled argon plasma spectroscopy (model 9000, Thermo Jarrell Ash, Waltham, MA) as described by Li *et al.* [7]. Muscular Insulin receptor, Insulin receptors phosphorylation, insulin receptor substrate-1, serine/thereonine kinase. phosphoinositide-3-kinase and glucose transporter protein 4 were determined using ultra sensitive chickens ELISA kits (Cat. No E-EL-ch 1110, Elabscience, Beijin; KHR9121, Invitrogen, USA; KT-56519, Kamiga biomedical, USA; JM-K453-40,MBL, USA; E-EL-ch0531, Elabscience, Beijin and AMSE12G0201, AMSbio, UK.) respectively following the manufacturer instructions.

RNA isolation, reverse transcription, and polymerase chain reaction:

Total RNA was prepared from the frozen muscular powder using the E.Z.N.A ™.spin column RNA extraction kit (Omega Bio-Tech, Cat NO R6834-01, Canada) following the manufacturer instructions. Concentrations of RNA were measured by spectrophotometry (OD 260 nm), and RNA integrity was electrophoretically verified using ethidium bromide. After DNAse treatment (Ambion, Clinisciences, Montrouge, France), RNA was reverse transcribed using Super Script II RNase H Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) in the presence of Random Primers (Promega, Charbonnièresles- Bains, France). Polymerase chain reaction (PCR) was performed using a 2720 thermocycler (Applied Biosystems, USA). Using PCR master mix (Qiagen USA) following the manufacturer instructions and using the specific primer (Table 2). PCR products were analyzed on a

2% agarose gel in 90 mM Trisborate, 2 mM EDTA buffer (TBE), pH 8, and visualized by staining with ethidium bromide and UV transillumination, For quantitative evaluation, absolute optical densities (OD) of RT-PCR signals were obtained by densitometric scanning using an image analysis system (1-D Manager; TDI Ltd.). The values for the specific targets were normalized according to those of β actin to express arbitrary units of relative abundance of the specific messages (i.e., relative expression).

Statistical analysis:

The data were statistically analyzed by SPSS version 20. statistical packages (IBM 1 New Orchard Road Armonk, New York 10504-1722 United States). Data were presented as a mean ± SD, n = 10. Statistical differences between groups were performed using student's t-test. Differences considered significant when $p < 0.05$ [14].

Table 2: primers used for polymerase chain reaction:

Gene	Primer sequence		Product size bp	Annealing (°C)	Accession No	Reference
IR	F	5\ TTTGGGATGGTTTATGAGGG 3\	383	58	XM_001233398.1	[2]
	R	3\GCCAGGTCTCTGTGAACAAA 5\				
IRS1	F	5\ GCCCGGCCCACGAGGCTG 3\	490	58	NM_001031570.1	
	R	3\ GTACGCTTGTCCGTAACG 5\				
Bactin	F	5\ AGCCATGTACGTAGCCATCC3\	230	55	NM_ 205518.1	Afifi and Alkaladi 2011
		5\ CTCTCAGCTGTGGTGGTGAA3\				

Results:

Table 3: Effect of Zn deficiency and supplementation on serum glucose, serum insulin, muscular glycogen and pancreatic Zn.

Group	Blood glucose (mg/dl)	Serum insulin (ng/ml)	Glycogen (mg/kg)	Pancreatic Zn(µg/ml cytosol)
I	275 ± 13.2	0.76 ±0.07	53.7 ± 4	15.7 ± 2.5
II	486.6 ± 7.6^{a}	0.25 ± 0.05^{b}	27.7 ± 2.5^{b}	10.3 ± 2^{b}
III	225 ± 5^{fg}	0.58 ± 0.8^{gk}	47 ± 3.6^{fh}	26.3 ± 1.5^{fh}

a,b,c represent the statistical difference of group II relative group I at □ □ (0.001, 0.01 and 0.05) respectively. d,e,f represent the statistical difference of group III relative group I at □ □ (0.001, 0.01 and 0.05) respectively. g,h,k, represent the statistical difference of group III relative group II at □ □ (0.001, 0.01 and 0.05) respectively.

Table 4: Effect of Zn deficiency and supplementation on muscles insulin signals

G	IR (ng/ml)	IRP (ng/ml)	IRS (ng/ml)	AKT (ng/ml)	PI3K (ng/ml)	GLUT4 (ng/ml)	IR gene expression (arbitrary unit)	IRS1gene expression (arbitrary unit)
I	23 ± 2.6	4.3 ± 1.2	33.3±1.5	2.2 ± 0.3	16.3 ± 1.5	2.5 ± 0.2	3.1 ± 0.62	11.3 ± 1.32
II	25 ± 6.1	2.5 ± 0.5^{c}	32±2	1.5 ± 0.2^{c}	6.3 ± 1.5^{c}	1.3 ± 0.3^{c}	2.9 ± 0.71	10.6 ± 1.22
III	21 ± 2.1	5.3 ± 0.8^{k}	34.7±1.5	3.2 ± 0.3^{fh}	25.3 ± 2.5^{fh}	4 ± 1^{k}	3.2 ± 0.42	12.3 ± 2.45

G; group. IR; insulin receptor. IRP; insulin receptor phosphorilation. IRS; insulin receptor substrate-1. AKT; serine/thereonine kinase. PI3K; phosphoinositide-3-kinase. GLUT4; glucose transporter protein 4. a,b,c represent the statistical difference of group II relative group I at □ □ (0.001, 0.01 and 0.05) respectively. d,e,f represent the statistical difference of group III relative group I at □ □ (0.001, 0.01 and 0.05) respectively. g,h,k, represent the statistical difference of group III relative group II at □ □ (0.001, 0.01 and 0.05) respectively.

Effect of either Zn deficiency or supplementation on serum glucose, muscular glycogen, serum insulin and pancreatic cytosolic Zn concentrations:

Zinc deficiency in chickens accompanied with a significant increase of blood glucose (□ □ 0.001), decrease of muscular glycogen, serum insulin and pancreatic cytosolic zinc concentrations (□ □ 0.01). In contrast Zn supplementation to chicken significantly decrease blood glucose and increase Muscular glycogen, serum insulin and pancreatic cytosolic Zn concentrations, when either compared to control or Zn deficient chicks (table 3).

Effect of either Zn deficiency or supplementation on muscular insulin signal molecules:

Either Zn deficiency or supplementation not significantly affect on either concentrations or gene expression of both IR and IRS-2. While Zn deficiency significantly decreases the concentrations of muscular IRP, AKT, PI3K and GLUT4, Zn supplementation significantly increase the above mentioned parameters ..

Discussion:

Chicken rearing nowadays becomes a high established manufacture due to the growing high demands of a ship protein, that can be get from the high growth rat chicken. The main column of this manufacture is the diet, that mainly a carbohydrate dependant "the carbohydrates metabolism mainly controlled by insulin hormone". In mammals , insulin synthesis, storage, secretion and signaling modulated by Zn status but that not established in chickens. This work is a trial to know the modulatory effect of Zn status on insulin synthesis and insulin signals in chickens.

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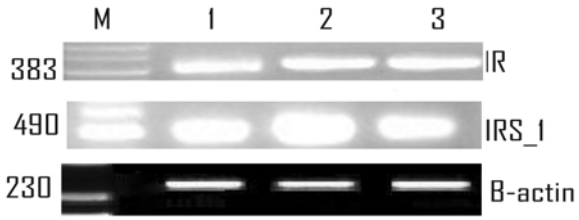

Fig. I: The expression level of mRNA for IR, IRS-1 and Betactin, M; DNA marker, 1; control group, 2 Zn deficient group, 3; Zn supplemented group.

The current results indicated that, In contrast to Zn supplemented chickens, Zn deficient chickens showed a decrees in pancreatic Zn, Serum insulin, Liver glycogen and increase in blood glucos. Indeed the results are correlated and explain each other. The decrease in pancreatic cytosol Zn concentration related to Zn deficiency in diet, where pancreas contains large amount of Zn and is the first organ affected by Zn deficiency. The decrease of serum insulin in Zn deficient group and it's increase in Zn supplemented one indicates the importance of Zn in regulation of serum insulin level , this may be through regulating insulin gene expression, or insulin modification, or storage, or excretion or may be all this processes. Insulin is important for entrance of glucose to hepatic cells and glycogen synthesis this explain the increase of blood glucose and the decrease of hepatic glycogen concentration. The above explanations are enforced by the results obtained in Zn supplemented group where, the Zn supplementation disappear all Zn deficiency effects, this indicated that, Zn is the cause of this effects (table 3).

The total Zn^{2+} content of the mammalian pancreas is high, and these ions are chiefly localized to the islet β-cell . Correspondingly, Zn^{2+} plays an important role in both insulin synthesis and storage. Indeed, total Zn^{2+} concentrations reach millimolar levels in the interior of the dense-core granule , where two Zn^{2+} ions coordinate six insulin monomers to form the hexameric structure on which insulin crystals are based [3]. It has been reported that pancreas is the most sensitive soft tissue to dietary Zn for chicks, and pancreas Zn concentration was shown to be a useful indicator for Zn requirement of broilers [5,13] reported that, in contrast to Zn supplementation, *db/db* mice fed the low-Zn diet had higher serum fasting glucose (17%) and lower serum fasting insulin (63%) concentrations than *db/db* mice fed the Zn-adequate diet . The interactions among Zn, insulin, and glucose homeostasis are complex, and Zn deficiency might induce a state of insulin deficiency by interfering with either insulin storage or activation [8].

Either Zn deficiency or supplementation not affect on IR and IRS-1 gene expression and concentrations, but IRP, PI3P, KAT and GLUT4 were inhibited by Zn deficiency and activated by Zn supplementation (taple 4 and fig 1). This indicts that, Zn not affect on action of insulin on insulin receptors but its action apeare postreceptor either through activation of receptor tyrosine kinase phosphorylation or Activation of PI3K/KAT pathway leading to activation of GLUT4 that increase the entrance of glucose to muscle cells. Several modes of action have been described to explain the improved action of insulin by Zn. It appears that

Zn can have direct insulin-like effects, which may be due to stimulation of the postreceptor proteins Akt and PI3-kinase [10] Several potential mechanisms have been suggested for Zn affecting insulin action, including a role for Zn to enhance tyrosine kinase phosphorylation [13].

Some of the insulinomimetic effects of zinc can be explained by the induction of translocation of GLUT to the plasma membrane, through activation of one zinc-dependent molecule, insulin-responsive aminopeptidase (IRAP), which is expressed and characterized in fat and muscle as insulin target tissues, resulting in an increased uptake of glucose into tissue cells, thereby lowering the blood glucose level [11].

Like insulin, zinc enhances glucose uptake into fibroblasts and adipocytes, which suggests an involvement of zinc in this pathway. Examining the effects of zinc on the insulin signal transduction, it was observed that zinc leads to tyrosine phosphorylation of the β subunit of the insulin receptor, but to a lower extent compared to insulin, and that IRS does not seem to play a role in enhancing glucose uptake as a response to zinc stimulus. According to this model, which proposes an activation of PI3Kwithout involvement of IRS, zinc may induce the production of H2O2 by epididymal cells, which in turn causes the activation of focal adhesion kinase (FAK) and FAK can finally activate the PI3K-Akt pathway [11].

Support for the involvement of zinc in phosphorylation of the insulin receptor was provided by Haase and Maret [4] who identified PTP1B as a sensitive target of zinc ions and an important regulator of the phosphorylation state of the insulin receptor. Inhibition of PTP1B by zinc ions, which might be released from Metalothionine (MT), leads

to an increased phosphorylation status of the insulin receptor triggering the post-receptor events. Considering that oxidative stress leads to a release of zinc from MT and to cellular zinc depletion, this condition as well as zinc deficiency due to decreased absorption, increased excretion or increased requirements could possibly lead to diabetes mellitus Furthermore, zinc increased phosphorylation of serine residues and therefore activation of Akt in preadipocytes and adipocytes thereby enhancing GLUT translocation. This effect could be blocked by wortmannin, an inhibitor of PI3K, underlining the importance of PI3K for the activation of Akt by zinc [13].

Conclusion:

It can be concluded that, like mammals Zn activate ß cells for production of insulin, and increase insulin signals in muscle through activation of PI3K-AKT pathway and GLUT4. So it play important role in glucose homeostasis in chickens.

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