

## EFFECTS OF HYPOMAGNESEMIA ON NITRIC OXIDE, ERYTHROCYTES ANTIOXIDANT ENZYMES DEFENSE SYSTEM AND SOME HORMONES IN GOATS

BY

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### SUMMARY

*This study aimed to investigate the effect of hypomagnesemia on glutathione redox cycle, Nitric oxide, serum cortisone and insulin. Twelve healthy Baladi male goats were used in this study. They were divided into 2 groups; the first one contained 5 animals and kept as control healthy group. The other group contained 7 animals subjected to experimental hypomagnesemia by daily oral administration of potassium chloride and citric acid until development of the characteristic signs of hypomagnesemia. Blood samples were collected at 6, 12, 18 and 24 days of administration .The recorded data revealed that serum magnesium concentration decreased in experimental group compared with the values of control group. There was a significant decrease in serum nitrate, and glutathione peroxidase (GSH-Px), glutathione (GSH), glutathione reductase (GR-ase), glutathione S-transferase (GST) and total superoxide dismutase (t-SOD) activities, whereas catalase (CAT) activity was significantly increased in hypomagnesemic group compared to the control group. Serum insulin levels decreased significantly, whereas serum cortisone levels increased in comparison to the values in the control healthy group. From these results we can conclude that nitric oxide release, glutathione redox cycle activities, insulin and cortisone levels were markedly affected by hypomagnesemia. These changes may be the main causes of tissue injury and vascular changes that usually accompanying hypomagnesemia.*

### INTRODUCTION

Magnesium is the most abundant intracellular ion; it activates many enzymes involved in the central pathway of cellular metabolism. Glycolysis, oxidative phosphorelation, nucleotide

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metabolism and protein synthesis are magnesium - dependent processes (*Bronzetti et al. 1995*). Magnesium influences physico-chemical properties of cellular membranes, thus is involved in establishing and maintaining intracellular electrolytes content. Also it acts as cofactor for multiple enzymes and its severe deficiency induces oxidative damage by increasing the production of reactive oxygen species (ROS) and enhancing susceptibility to oxidative stress and changes in antioxidant status (*Wiles 1997*). Extracellular magnesium plays a major role in acetylcholine production and destruction; also it is found in neuromuscular junction (*El-Sayed et al. 2002*). Therefore hypomagnesemia is accompanied with neuromuscular disturbances and tetany (*Aboul-Seoud 2000*).

Nitric oxide (NO) induces vasodilatation, regulates normal vascular tone and inhibits platelets aggregation (*Moncada and Higgs 1995*). Moderate magnesium deficiency during pregnancy affects blood pressure and NO production and release from coronary epithelium (*Carlin and Franz 2002*). Also impairment of glutathione peroxidase inhibits NO production by endothelial cells (*Upchurch et al. 1997*).

The frequency of hypomagnesemia is high in diabetic patients and it is correlated with the severity of hyperglycemia. Hypomagnesemia affects insulin through changing its sensitivity or secretion (*Tosiello 1998*).

The glutathione redox cycle composed of GSH-Px and GR-ase and the co-substrates GSH and NADPH. Intracellular GSH and GSH-dependent enzymes provide cellular protection against oxidant by using peroxidase to reduce hydrogen or lipid peroxide with the concurrent oxidation of GSH to glutathione disulfide (GSSH), which reduced back to GSH by GSSG-R through the reducing power of NADPH provided by pentose phosphate pathway. In addition GSH is co-substrate for detoxification of some toxic agents by glutathione S-transferases and also may scavenge ROS including hydroxyl radical, singlet oxygen, NO and peroxynitrite (*Halliwell and Gutteridge 1998*).

Hypomagnesemia increased the activity of catalase enzyme in cardiac muscle, while the activity of GSH-Px was decreased significantly (*Kuznair et al. 2001*). Alteration in the activities of the antioxidant enzymes was suggested to be responsible for cardiac muscle lesions observed in hypomagnesemia (*Kury et al. 2001*).

Therefore this study was designed to investigate the possible effects of hypomagnesemia on NO release, activities of antioxidant enzymes of glutathione redox cycle, insulin and cortisone hormones in Goats.

## MATERIALS AND METHODS

### **Animals:**

The present study was carried out on the farm of Faculty of Veterinary Medicine (Moshtohor) on 12 apparently healthy adult male Baladi goats, 9-21 months old and their body weight ranged between 28-32 kg. The animals were clinically normal and free from any external, blood and internal parasites, they were fed a constant diet composed of barseem (*Trifolium alexandrium*) and a pelleted concentrate mixture consisted of the following ingredients: bran 40 %, corn 22 %, cotton seed cake 35 %, calcium carbonate 2 %, and sodium chloride 1 %. The diet composition was 14 % crude protein, 15 % fibers and 3 % fat. About 0.5 kg/animal of concentrate was distributed twice a day in addition to 2 kg barseem. Water was offered *ad libitum*. Animals were divided into two groups: *Group-A*: five animals were used as control group. *Group-B*: seven goats were used as experimental group for the induction of hypomagnesemia by daily oral administration of potassium chloride {Sigma chemical company as Pure crystalline MW.74.55} and citric acid {Sigma chemical company as (anhydrous free acid) crystalline MW.192.10} at a dose of 1.39 g/kg and 1.19 g/kg body weight respectively for 24 days by using the stomach tube according to Hazarika and Pandey (1993) and Hefnawi (2000).

### **Biochemical assays:**

The blood samples were collected at 6, 12, 18 and 24 days after starting of administration from the jugular vein of both groups and divided into two parts. The first part was collected without anticoagulant for separation of serum samples, which were used freshly for the quantitative determination of nitrate concentration (which is the stable end product of nitric oxide). Nitrate was determined by an end point one-step enzymatic assay with nitrate reductase as described by Bories and Bories (1995). Serum cortisone and insulin concentrations were estimated by using immunoradiometric assay (IRMA) according to Wilson and Miles (1977) and Mullner et al. (1991). Mg was measured by the method adopted before by Bohuon (1962). The second part of blood samples was collected in tubes contained 20 IU heparin / 1ml blood; and used for preparation of hemolysate by Digitonine after washing erythrocytes by physiological saline as described by Kornburg and Korecker (1955). This hemolysate was subjected for quantitative determination of erythrocytic GSH-Px (EC1.11.1.9 )

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(Chiu et al. 1976); GSH ; GR-ase (EC1.6.4.2) and GST(EC 2.5.1.18) ( Bergmayer 1983); t-SOD ( EC 1.15.1.1) ( Misra and Fridovich 1972 ) and catalase ( EC 1.11.1.6 ) (Sinha 1972).

**Statistical analysis:**

Data were expressed as mean  $\pm$  SE. Differences between groups were examined for statistical significance using the Student's *t*-test as explained by *Petrie and Waston (1999)*.

## RESULTS

**Clinical symptoms:** The symptoms of hypomagnesemia were appeared gradually and includes loss of appetite, bellowing alertness, extension of fore limbs, unusual movement, depression, grinding of teeth, laying down and getting up frequently, tetany, staggering gait and convulsions in the form of episodes with hyperesthesia.

**Serum parameters:** the results of the changes in the serum parameters including, Mg, nitrates, insulin and cortisone are showed in Table (1). A significant decrease ( $P < 0.05$ ) was recorded in the serum Mg level during 6<sup>th</sup>, 12<sup>th</sup> and 18<sup>th</sup> day from the beginning of the experiment; and high significant decrease ( $P < 0.01$ ) during 24<sup>th</sup> day. Serum nitrate values decreased significantly ( $P < 0.05$ ) during 12<sup>th</sup> day; high significant decrease ( $P < 0.01$ ) during 18<sup>th</sup> day and very high significant decrease ( $P < 0.001$ ) during 24<sup>th</sup> day were found compared to control group. Cortisone levels showed high significant increase ( $P < 0.01$ ) during 12<sup>th</sup> and 18<sup>th</sup> day and very high significant increase ( $P < 0.001$ ) during 24<sup>th</sup> day of the experiment; while insulin level decreased significantly ( $P < 0.05$ ) during 12<sup>th</sup>, 18<sup>th</sup> and 24<sup>th</sup> day in comparison to the values of the control group.

**Glutathione redox cycle:** data in table (2) showed significant decrease ( $P < 0.05$ ) in GSH-Px, GSH, GR-ase, GST and t-SOD; whereas there was a high significant increase ( $P < 0.01$ ) in the activities of catalase during 18<sup>th</sup> and 24<sup>th</sup> day of the experiment in comparison to the values of the control group.

## DISCUSSION

Magnesium is a key ionic modulator of blood vessel wall. It reduces the development of hypertension induced by NO inhibitors (Manzo *et al.* 2002).

In this study, Hypomagnesemia was experimentally induced in goats by administration of high amount of potassium chloride and citric acid. The excessive amount of potassium chloride and citric acid in the rumen resulted in impairment of Mg absorption from the reticulorumen (Hefnawi 2000 and Radostits *et al.* 2000).

Results in our study revealed marked decrease in NO production in hypomagnesemic goats. These results are in accordance with Carlin and Franz (2002) who observed that moderate Mg deficiency during pregnancy adversely affect NO production and blood pressure. Pearson *et al.* (1998) reported that although hypomagnesemia inhibits NO release from coronary

produce NO, but selectively disrupts the signal transduction pathway leading to production of NO. Decreased production of NO Could also attributed to the decreased insulin level in hypomagnesemic goats as suggested by Bhattach *et al.* (2001) who reported that insulin activates NO synthase, which is required for NO synthesis. The decreased activity of GSH-Px in hypomagnesemic goats might be another explanation for the decrease in NO production. This is confirmed by Upchurch *et al.* (1997) who found that impairment of resulted in inhibition of NO production from endothelial cells.

Insulin level showed significant decrease in hypomagnesemic goats, these results are similar to that reported by Tosiello (1998) who found that Mg deficiency aggravated insulin resistance and its supplementation improve insulin sensitivity and secretion. Ruckebusch *et al* (1991) also reported hypomagnesemic cow have hyperglycemia without increase in insulin level. The severity of hypoinsulinemia and hyperglycemia in diabetic patients are correlated with hypomagnesemia (De Valk *et al.* 1998).

The observed increased values of serum cortisone levels in the present study are in agreement with the data of Mizushima *et al.* (1998) who noticed that Mg deficiency induced vascular damage, vasoconstriction of the coronary arteries, increased blood

pressure and increased cortisone level. These increased values could be related to the stressful conditions due to hypomagnesemia. This is because Mg normally affects the limbic-hypothalamus and pituitary-adrenocortical axes reducing the release of adrinocortico-tropic hormone (ACTH) and affecting the adrinocortical sensitivity to ACTH (Murck 2002).

Biochemical parameters showed significant decrease in the activities of t-SOD, GSH-Px, GSH, GR-ase and GST, while catalase enzyme was high significantly increased; these results are in accordance with Rude (1993). The oxidative stress induced by hypomagnesemia includes a disturbance between the pro-oxidant and antioxidant balance in favor of the former, which contributed to the pathologic effects observed in hypomagnesemia (Whang et al. 1994). Hypomagnesemia also increase oxygen radical concentration after its transformation to H<sub>2</sub>O<sub>2</sub> by stimulated neutrophils and macrophages; this leads to strong oxidation of cystein moiety of antioxidant enzymes decreasing its activity (Sarker et al. 1998).

Mg ion is required in the processes of transcription and replication of the antioxidant enzymes; this leads to decreased activity of antioxidant enzymes except catalase incase of hypomagnesemia (Kuznair et al. 2003). Catalase enzyme showed high significant increase in hypomagnesemic goats; The elevation in catalase activity indicates adaptation changes in response to large amount of hydrogen peroxide, which decomposed by catalase (Kumar and Shivakumar 1997). The significant decrease in the activity of SOD, GSH-px, GSH,GR-ase and GST reported in our study leads to depletion of antioxidant defense mechanism in RBCs, which cause inability of RBCs to remove harmful effects of arising O<sub>2</sub> in hypomagnesemia.

In conclusion, the tissue injury and vascular changes accompanying hypomagnesemia may be due to reduction of NO release and decreased in the activity of glutathione redox cycle.

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Table (1): Changes in serum parameters in control and experimental hypomagneseemic goats.

	Control	Hypomagneseemic animals			
		6 days	12 days	18 days	24 days
Nitrate (umol/L)	1.56	1.03	1.20	1.17**	0.84***
Magnesium (mg/dl)	0.26	0.31*	1.17*	0.13*	0.03**
Insulin (uU/ml)	0.22	0.38	0.81*	1.01*	0.23*
Cortisone (Ng/ml)	0.06	0.19	0.99**	1.01**	0.79***

N= 7.

P values: significant \* $<0.05$ , high significant \*\* $<0.01$  and very high significant \*\*\* $<0.001$ .

Table (2): Changes in glutathione redox system in control and experimental hypomagneseemic goats.

	Control	Hypomagneseemic animals			
		6 days	12 days	18 days	24 days
GSH-Px (U/gm protein)	0.89	0.79	0.10	0.05*	0.03*
GR-ase (U/gm protein)	0.01	0.11	0.12	0.06	0.01*
GST (U/gm protein)	0.09	0.13	0.11	0.02*	0.13
GSH (U/gm protein)	0.07	0.12	0.10	0.03*	0.01*
t-SOD (U/gm protein)	12.10	1.12	1.00	0.91*	0.75*
CAT (U/gm protein)	24.61	2.10*	1.91**	3.14***	5.71***

N= 7.

P values: significant \* $<0.05$ , high significant \*\* $<0.01$  and very high significant \*\*\* $<0.001$ .

## المخلص العربي

### تأثير نقص الماغنسيوم على أكسيد النيتريك ونظام دفاع إنزيمات مضادات الأكسدة للخلايا الدموية الحمراء وبعض الهرمونات في الماعز

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تم إجراء هذا البحث لدراسة تأثير نقص الماغنسيوم على أكسيد النيتريك و نظام الجلوتاثيون ريدوكس و مستوى الكورتيزون و الأنسولين في الماعز البلدي و قد استخدم لهذا الغرض اثني عشر من الذكور تم تقسيمهم الى مجموعتين الأولى الضابطة تحتوى على خمسة حيوانات سليمة بلا تدخل في غذائها - المجموعة الثانية تحتوى على السبعة الباقية تم إحداث فيها نقص الماغنسيوم تجريبيا بتجريعهم كلوريد البوتاسيوم و حمض الستريك (1.39 جم/كجم و 1.19 جم/كجم بالترتيب) - بعد ظهور الاعراض المميزة لهذا المرض تم تجميع عينات الدم بعد 6, 12, 18, و 24 يوما من إحداث هذا النقص و تم قياس النيترات و الكورتيزون و الانسولين في مصل الدم و الجلوتاثيون بيروكسيد و الجلوتاثيون المختزل و الجلوتاثيون ريدكتيز و الجلوتاثيون-S - ترانسفيريز و السوبر اكسيد ديسميوتيز الكلى و الكتاليز في خلايا الدم الحمراء و قد أظهرت النتائج نقصا معنويا في نشاط الجلوتاثيون بيروكسيد و الجلوتاثيون المختزل و الجلوتاثيون ريدكتيز و الجلوتاثيون-S - ترانسفيريز و السوبر اكسيد ديسميوتيز الكلى بينما ازداد نشاط الكتاليز زيادة معنوية - كما أظهرت النتائج انخفاض في مستوى الانسولين و ارتفاع في مستوى الكورتيزون في المجموعة المحدث فيها نقص الماغنسيوم بالمقارنة بالمجموعة الضابطة - و من هذه النتائج يمكن استخلاص ان نقص الماغنسيوم قد اثر في نشاط نظام الجلوتاثيون ريدوكس و إنتاج أكسيد النيتريك و مستوى الانسولين و الكورتيزون في الدم

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