Myanmar Health Sciences Research Journal, Vol. 29, No. 1, 2017

Serum and Erythrocyte Magnesium and Insulin Sensitivity in Adult Lean and Obese Subjects

Nway Htike Maw[1], Cho Lwin Aung[2], Mya Mya Thwin[2] & Ohnmar[3]*

[1]Department of Medical Research
[2]University of Medicine 2 (Yangon)
[3]University of Medicine 1(Yangon)

Magnesium is required for both proper glucose utilization and insulin signaling. Alterations in cellular magnesium may occur in obesity and it contributes to insulin resistance. So, this study aimed to evaluate the relationship between serum or erythrocyte magnesium level and insulin sensitivity and beta-cell function of adult male subjects. A total of 103 apparently healthy adult male subjects between 18-60 years of age participated in this study. The subjects were divided into two groups according to BMI (obese group: BMI≥23 kg/m^2 and lean group: BMI<23 kg/m^2). Serum magnesium and erythrocyte magnesium levels of subjects were assessed by colorimetric method. Insulin sensitivity and beta-cell function were assessed by homeostasis model assessment method (HOMA) based on fasting blood glucose and fasting insulin level. Oxidase method was used to assess blood glucose and enzyme-linked immunosorbent assay for fasting insulin. The mean erythrocyte magnesium level of the obese subjects (n=52) (4.98±0.68 mg/dl) was significantly lower than that of the lean subjects (n=51) (5.39±0.84 mg/dl). In this study, no relationship was found between serum or erythrocyte magnesium and HOMA-IR and HOMA-β cell in both obese and lean subjects. Therefore, this study could not find out the significant relationship between serum magnesium and erythrocyte magnesium with insulin resistance (HOMA-IR) and beta-cell functions (HOMA-β) in healthy adult male subjects.

Key words: Erythrocyte magnesium, Insulin sensitivity, Obese and lean subjects

INTRODUCTION

Magnesium is the second most abundant intracellular cation and is a cofactor of many enzymes involved in glucose metabolism.[1-3] It has an important role in insulin action and insulin also stimulates magnesium uptake in insulin-sensitive tissues.[4] Insulin sensitivity is the ability of insulin to exert its physiological action.

When insulin binds insulin receptor, the receptor tyrosine kinase is activated and it promotes autophosphorylation of the receptor β subunits. A sequence of reactions follow and the most important endpoint is the facilitated entry of glucose into the cell.[4] Dysregulation at many steps may lead to insulin resistance.[5, 6] Moreover, magnesium has been described as nature's physiologic calcium blocker and it regulates the entry of calcium to cells. A poor intracellular magnesium concentration may result in a defective tyrosine kinase activity and exaggerated intracellular calcium concentration which inhibits insulin receptor dephosphorylation in adipocyte. Both events are responsible for the impairment in insulin action and worsening of insulin resistance. Generally, insulin secretory activities in fasting state as well as glucose stimulated state reflect beta-cell function. Initially,

*To whom correspondence should be addressed.
Tel: +95-95056600
E-mail: nwayhtikemaw@gmail.com

insulin resistance can be overcome by increasing insulin secretion, but beta-cell failure eventually leads to diabetes.[5, 6] Insulin sensitivity declined with increasing body mass index (BMI).[7] So, obese individuals develop resistance to the cellular actions of insulin, characterized by an impaired ability of insulin to inhibit glucose output from the liver and to promote glucose uptake in fat and muscle.[8] Accumulating body of evidence demonstrated that magnesium deficiency is linked with deranged glucose metabolism and insulin resistance. Insulin resistance is etiological factor for type 2 diabetes mellitus.[9, 10]

The burden of diabetes is increasing globally, particularly in developing countries. The role of micronutrients such as calcium and zinc on insulin action has also been extensively studied in normal as well as diabetes patients, but the role of magnesium in insulin action and proper glucose utilization is needed to point out. Therefore, this study aimed to find out the relationship between serum and erythrocyte magnesium with insulin sensitivity and beta-cell function in adult normal male subjects.

MATERIALS AND METHODS

Study population

A cross-sectional analytical study was conducted in apparently healthy males, 51 lean subjects and 52 obese subjects aged between 18-60 years residing in North Okkalapa Township. Exclusion criteria included acute illness, history of diabetes mellitus (i.e., fasting blood glucose >120 mg/dl), chronic renal disease (i.e., serum creatinine >1 mg/dl) and history of taking drugs that affect magnesium metabolisms (eg. diuretic, magnesium-containing antacids).

Written informed consent was taken from all participants. Weight, height and waist circumference were measured. Body mass index was calculated and expressed as weight in kg per height in m^2.

Determination of serum magnesium

Serum magnesium concentration was determined by using Calmagite method.[11, 12] One millilitre of blood was collected in a metal-free container without anti-coagulant. Red cells were separated immediately. Unhaemolysed serum was collected to determine magnesium using Spectrophotometer.

Determination of erythrocyte magnesium

Erythrocyte magnesium concentration was determined by using Calmagite method.[11, 12] Two millilitres of blood were kept in the metal-free container containing heparin and centrifuged. After elimination of plasma, erythrocytes were washed 3 times with saline solution and then, the supernatant was eliminated. Erythrocyte magnesium was determined by using Spectrophotometer.

Assessment of insulin sensitivity and β-cell function

Serum fasting glucose was measured by glucose oxidase method. Serum insulin level was measured by enzyme-linked immunoassay (ELISA) method. Insulin sensitivity and β-cell function were calculated by following formulae.

The HOMA index of insulin resistance (IR) was calculated as follows:

$$\text{HOMA-IR} = \frac{[\text{Insulin } (\mu\text{IU/ml}) \times \text{Glucose (mmol/l)}]}{22.5}$$

The HOMA index of insulin secretion (β-cell function) was calculated as follows:

$$\text{HOMA } \beta\text{-cell function} = \frac{20 \times \text{Insulin } (\mu\text{IU/ml})}{\text{Glucose (mmol/l)} - 3.5}$$

Statistical analysis

All data were presented as mean±SD. Pearson's correlation was used to assess the relationship between serum and erythrocyte magnesium level and insulin sensitivity and beta-cell function of adult male subjects. Statistical significance was set as p values of less than 0.05. Independent student '*t*' test was used to compare the continuous variables.

RESULTS

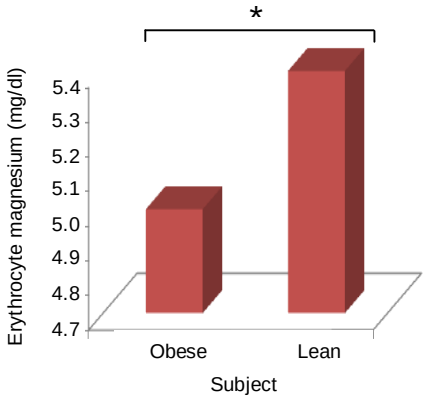
This study included 52 obese subjects and 51 lean subjects. General characteristic of subjects are shown in Table 1.

Table 1. General characteristics of the obese and lean subjects

	Obese subjects (n=52)	Lean subjects (n=51)
Age (years)	35.1±13.44	34.61±13.04
BMI (kg/m^2)	27.07±3.78*	19.95±1.97
WC (cm)	90.33±10.32*	71.32±6.51

*indicates significant difference between two groups ($p<0.05$)

Mean serum magnesium level of the obese subjects (1.6±0.27 mg/dl) was comparable with that of lean subjects (1.68±0.27 mg/dl). These serum magnesium levels of both groups fell within the normal magnesium level (1.6-2.6 mg/dl). However, the mean erythrocyte magnesium of obese subjects (4.98±0.68 mg/dl) was significantly lower than that of lean subjects (5.39±0.84 mg/dl) (Fig. 1).



*indicates significant difference between two groups ($p<0.05$)

Fig. 1. Erythrocyte magnesium level in obese and lean subjects

The fasting glucose was not significantly different between obese and lean subjects (5.51±1.03 mg/dl and 5.16±1.29 mg/dl) but serum fasting insulin level of obese subjects (12.88±7.48 µIU/l) was significantly higher than that of lean subjects (7.51±6.15 µIU/l). Calculated values of HOMA-IR and HOMA-β of the both groups are shown in Table 2 and these values were significantly higher in the obese subjects than the lean subjects.

Table 2. Fasting blood glucose and fasting insulin, insulin resistance (HOMA-IR) and beta-cell function (HOMA-β cell function) in obese and lean subjects

	Obese subjects (n=52)	Lean subjects (n=51)
Fasting glucose (mmol/l)	5.51±1.03	5.16±1.29
Fasting insulin (µIU/l)	12.88±7.48*	7.51±6.15
HOMA-IR	3.17±1.9*	1.79±1.6
HOMA-beta	151.52±117.33*	109.05±75.94

*indicates significant difference between two groups ($p<0.05$)

The results of the present study found no correlation between serum magnesium or erythrocyte magnesium and HOMA-IR and HOMA-β (Table 3).

Table 3. Relationship between erythrocyte magnesium, serum magnesium and HO-MA-IR and HOMA-β cell function in adult male subjects

Particular	r	p
Correlation between erythrocyte magnesium and HOMA-IR	0.07	NS
Correlation between erythrocyte magnesium and HOMA-β cell function	0.1	NS
Correlation between serum magnesium and HOMA-IR	0.09	NS
Correlation between serum magnesium and HOMA-β cell function	0.14	NS

NS=Not Significant

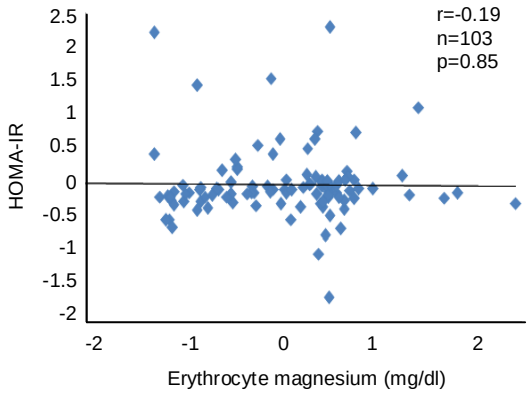


Fig. 2. Correlation between erythrocyte magnesium and HOMA-IR in adult male subjects (after adjusted the insulin)

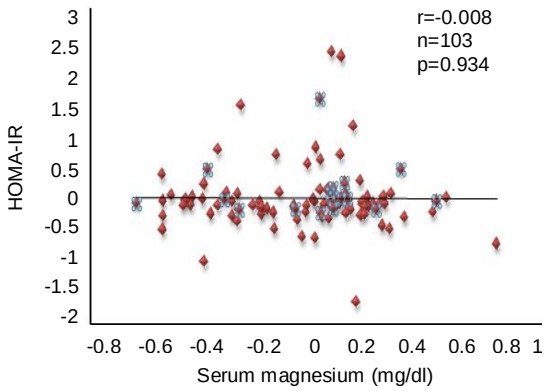


Fig. 3. Correlation between serum magnesium and HOMA-IR in adult males subjects (after adjusted the insulin)

However, when the effect of insulin was adjusted, both erythrocyte and serum magnesium were negatively correlated with HOMA-IR, but this correlation was not statistically significant (n=103, r=-0.19, p=0.85) (n=103, r=-0.008, p= 0.934) (Fig. 2 & Fig. 3).

DISCUSSION

The mean age of the participant of the obese and lean male subjects of the present study was 34.72±13.17 years. Generally, they had no signs and symptoms of nutritional deficiency. The serum magnesium levels of obese and lean subjects (1.6 ±0.27 mg/dl *vs.* 1.68±0.27 mg/dl) were within normal range (1.6-2.6 mg/dl). They were not significantly different. However, the mean erythrocyte magnesium level of obese subjects (4.98±0.68 mg/dl) was significantly lower ($p<0.05$) than that of lean subjects (5.39±0.84 mg/dl). This finding was in agreement with the findings of another study[13] in Myanmar. This study showed that the mean intracellular magnesium levels of centrally obese was significantly lower than the non-obese adult subjects. The reason why serum magnesium level of obese and lean subjects was not quite different in the present study was that the serum magnesium was highly regulated and the kidneys were the primary organ to regulate the magnesium homeostasis.[14]

In the present study, the subjects were selected based on serum creatinine level (≤1 mg/dl). The serum magnesium levels did not alter significantly even in the presence of magnesium deficiency in individuals with normal functioning kidneys.[15]

In this study, fasting glucose of all subjects (5.34±1.18 mmol/l) n=103 was within normal range (4.2-6.4 mmol/l). However, fasting plasma insulin level of obese subjects (10.22±7.33 μIU/l) was significantly higher than that of lean subjects (7.5±6.15 μIU/ml). This finding supported the concept that obesity is associated with hyperinsulinemia, decreased insulin sensitivity and increased β-cell function. Furthermore, insulin resistance (HOMA-IR) and beta cell functions (HOMA-β) of obese subjects (3.17±1.9 and 151.52±117.33) were significantly greater than those of lean subjects (1.79±1.6 and 109.05±75.94). It means that the beta-cell function might be increased to compensate the insulin resistance in obesity and the blood glucose level is well regulated in the obese subjects in expense of increased beta-cell activity.

The present study demonstrated that there were no relationship between serum and erythrocyte magnesium with insulin sensitivity (HOMA-IR) and beta-cell functions (HOMA-β) of all adult male subjects (n=103, Pearson's r=0.09, r=0.07, r=0.1, r=0.1, respectively). After the effect of insulin was adjusted, serum and erythrocyte magnesium were negatively correlated with HOMA-IR. These correlations were not statistically significant (n=103, r=-0.008, p=0.934, r=-0.19, p=0.85).

Some studies reported that magnesium deficiency might contribute to obesity associated insulin resistance. In the present study, there was no significant relationship between serum and erythrocyte magnesium with insulin sensitivity and beta-cell functions in obese and lean subjects. It might be due to small sample size and narrow variations of magnesium level between individuals in this study. Similar to

the present study, a study reported that neither serum nor intracellular magnesium was associated with HOMA-IR in obese and non-obese children.[16] They explained that insulin increases intracellular magnesium levels by stimulating entry of magnesium across the plasma membrane. Magnesium is required for glutathione biosynthesis which is the most abundant intracellular antioxidant. Thus, they suggested that the increase in intracellular erythrocyte magnesium was to compensate the increased oxidative stress in obesity. In the light of present study, it could be concluded that insulin sensitivity and beta-cell function were unaffected by intracellular magnesium level in healthy adult male subjects.

ACKNOWLEDGEMENT

we would like to express our deepest gratitude to Professor Dr. U Tint Swe Latt, Former Rector, University of Medicine 2, Yangon for his kind permission to conduct this study. We also deeply thank Professor Dr. Mya Thandar Sein, Department of Physiology, University of Medicine 2 for her guidance, advices and expert technical support, Dr. Ko Ko Zaw, Deputy Director, Epidemiology Research Division, Department of Medical Research for his help in statistical analysis and data management and Dr. Nwe Nwe Oo, Former Deputy Director, Biochemistry Research Division, Department of Medical Research for sharing their knowledge and method in implementing this research.

REFERENCES

1. Rude RK. Magnesium deficiency: A cause of heterogeneous disease in humans. *Journal of Bone and Mineral Research* 1998; 13: 749-758.
2. Bohl CH & Volpe SL. Magnesium and exercise. *Critical Reviews in Food Science and Nutrition* 2002; 42: 533-563.
3. Elin RJ. Assessment of magnesium status for diagnosis and therapy. *Magnesium Research* 2010; 23: 194-198.
4. Yenush L & White MF. The IRS- signaling system during insulin and cytokine action. *Bioassays* 1997; 19: 491-500.
5. Kasuga M, Zick Y, Blith DL, Karlsson FA, Haring HU & Kahn CR. Insulin stimulation of phosphorylation of the β subunit of the insulin receptor. *Journal of Biological Chemistry* 1982; 257(17): 9891-9894.
6. Pessin JE & Saltiel AR. Signaling pathways in insulin action: Molecular targets of insulin resistance. *Journal of Clinical Investigation* 2000; 106: 165-169.
7. Bogardus C, Lillioja S, Mott D, Reaven G, Kashiwagi A & Foley J. Relationship between obesity and maximal insulin stimulated glucose uptake *in vivo* and *in vitro* in Pima Indians. *Journal of Clinical Investigation* 1984; 73: 800-805.
8. Saltiel AR & Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* 2001; 414: 799-806.
9. Iseri LT & French JH. Magnesium: Nature's physiologic calcium blocker. *American Heart Journal* 1984; 108: 188-194.
10. Agus ZA, Kalipourie E, Dukes I & Morad M. Cytosolic magnesium modulates calcium channel activity in mammalian ventricular cells. *American Journal of Physiology* 1989; 256: 452-455.
11. Khayam-Bashi H, Liu TZ & Vern W. Measurement of serum magnesium with a centrifugal analyzer. *Clinical Chemistry* 1997; 23(2): 289-291.
12. Tietz NW. Philadelphia: WB Saunders. In: *Text Book of Clinical Chemistry* 1986; 971-975.
13. Thurien Zaw. Relationship of intracellualar magnesium level, serum magnesium level and congnitive function between centrally obese and non-obese adult subjects in Magway Township. *Thesis*, M.Med.Sc (Physiology) 2013, University of Medicine (Magway), Yangon.
14. Quamme GA & Dirks JH. The physiology of renal magnesium handling. *Renal Physiology* 1986; 9: 257-269.
15. Ryzen E, Elbaum N, Singer FR & Rude RK. Parenteral magnesium tolerance testing in the evaluation of magnesium deficiency. *Magnesium* 1985; 4: 137-147.
16. Tohidi M, Ghasemi A, Hadaegh F, Arbabi S & Hosseini FI. Intraerythrocyte magnesium is associated with gamma-glutamyl transferase in obese children and adolescents. *Biological Trace Element Reaearch* 2009; 143(2): 835-843.