Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.6, No.8, 2016

Influence of Magnesium as a Major Contributor of Water Hardness on Some Cardiac Disease Risk Factors

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Abstract
Various ecological studies report a reduction in cardiovascular disease mortality risk with increasing magnesium levels in drinking water. Most of the studies done in this field were epidemiologic studies.The aim of the present study was to examine whether magnesium addition to drinking water can affect risks of cardiac disease. The study included five groups of male albino rats. The rats received either tap water or water containing 5, 10, 20 g of magnesium sulfate per liter. During the whole experiment, all the groups received hypercholesterolemic diet except for the normal control which received normal basal diet. At the end of the experiment, blood was drawn for the determination of plasma magnesium, lipid profile and liver function. In addition, the extent of obesity was determined using the body mass index (BMI). In all groups magnesium addition was associated with higher levels of plasma magnesium. The blood analysis showed a significant decrease in serum total cholesterol, triglycerides, LDL- cholesterol and VLDL- cholesterol, while there was a significant increase in HDL-cholesterol in groups received magnesium sulfate in drinking water, compared with the hypercholesterolemic group received tap water. GOT, GPT and ALP followed the same trend. The addition of $MgSO_4$ to the drinking water results in significant decrease in BMI of the magnesium treated groups relative to the hypercholesterolemic control. These results showed that high magnesium concentration in drinking water is capable of decreasing some cardiac disease risk factors in male albino rats.
Keywords: Cardiac disease, hypercholesterolemia, Magnesium, Risk factors, Water.

1. Introduction

Water is essential for life and the consideration of the characteristics of water as determinants of disease risk is turning into a critical issue. Water hardness is among these characteristics. It is defined as the measure of the concentration of calcium and magnesium ions in water.

The bioavailability of Ca and Mg is accounted for to be higher from drinking water than from food (Couzy *et al.,* 1995; Durlach *et al.,* 1985). This is because Ca and Mg are normally present in water as free ions so, they are more readily absorbed compared to food, where they are usually bound to other substances (WHO, 2005).

The absorbability of Mg from water is higher by 30 % compared to dietary magnesium (Marx and Neutra, 1997).

Therefore, not to remove hardness components (Ca & Mg) from drinking water or in certain circumstances increasing the magnesium and / or calcium intake from water may be of great importance especially for populations with an inadequate dietary intake of these elements(WHO, 2009).

Many ecological investigations report a decrease in cardiovascular disease (CVD) mortality risk with increasing magnesium concentrations in drinking water and the evidence has accumulated that it is the main beneficial agent involved while the effect of calcium against CVD is only supportive. (Eisenberg, 1992).

Cardiovascular disease (CVD) is a class of disease that involves the heart or blood vessels (Mendis *et al.,* 2011).

Many important cardiovascular risk factors can be modified by changing lifestyle, social change, drug treatment and prevention of hypertension, hyperlipidemia, and diabetes mellitus. (Micha *et al.,* 2012).

The diet rich in cholesterol is a main contributor to unbalanced lipid metabolism and is associated with an increase in coronary heart disease occurrence. (Libby, 2008).

It was demonstrated by some studies that decreasing plasma total cholesterol, low- density lipoprotein cholesterol and increasing high-density lipoprotein cholesterol are of great benefit in preventing the risk of cardiovascular disease (Agbedana and Akanji, 1988).

The aim of the present study is to assess the interrelationship between high magnesium concentration in drinking water and some risks of cardiac disease.

2. Materials and Methods

2.1 Animals and diet

The animal protocol was designed to minimize pain or discomfort to the animals.

Thirty adult male albino rats weighed 120 -130 g were used in the present experiment. The rats were housed under controlled light and temperature conditions (12 hours light: dark cycle, 22-23°C) for two weeks.

The animals were kept in the animal house of Regional Center for Food and Feed in the agricultural research center, Giza, Egypt.

During this period, the normal diet (basal diet) as shown in table (1) and tap water were supplied ad lipitum. Each rat was weighed every week and the water intake was also recorded.

Table (1): The composition of the basal diet (Campbell, 1961).

Content	%
Casein	20
Corn oil	10
Salt mixture	4
Vitamins mixture	1
Cellulose	5
starch	60

Rats were divided into five groups each had 6 rats. The first group was fed on the basal diet and tap water and was considered as normal control (NC).

The other rats (24 rats) were fed on hypercholesterolemic diet for 12 weeks (10% sheep tail fat + 1 % pure cholesterol + 0.25 % bile salt).

They were divided into four groups.

The first group (6 rats) was fed on hypercholesterolemic diet + tap water and was considered as hypercholesterolemic control (HC).

The second group (6 rats) was fed on hypercholesterolemic diet + 5 g / L $MgSO_4$ added to tap water.

The third group (6 rats) was fed on hypercholesterolemic diet + 10 g / L $MgSO_4$ added to tap water.

The fourth group (6 rats) was fed on hypercholesterolemic diet + 20 g / L $MgSO_4$ added to tap water.

This hypercholesterolemic diet was shown to induce hypercholesterolemia in rats within three weeks.

2.2 Blood sampling

At the end of the experiment, blood samples were collected from each rat from the trirorbital venous plexus of the rat eye by capillary tube after an overnight fast, the blood samples were collected in dry tubes which were put in water bath at 37°c for 15 minutes, then centrifuged at 5000 r.p.m for 6 minutes and the colorless upper layers| (haemolysis – free serum) were carefully separated and transferred into sterilized test tubes for the analysis of total cholesterol, HDL- cholesterol, LDL- cholesterol, VLDL- cholesterol, triglycerides, as well as GOT, GPT, alkaline phosphatase activity (ALP) and serum magnesium.

Spectrophotometer of Microlab 200, Merck was used in all biochemical analysis and the reagents and the standards were ready to use.

2.3 Obesity assessment using the Body mass index

The body weight and body length were used to determine the Body mass index (BMI) (Novelli *et al.,* 2007).

Body mass index (BMI) = body weight (g) / length^2 (cm^2).

Body length (nose-to-anus or nose – anus length) was determined in all rats. The measurement was made in anaesthetized rats.

2.4 Statistical analysis of the data

Data were subjected to statistical analysis using the analysis of variance method and the means of treatments were compared by using the least significant difference (L.S.D) at 0.05 level of probability according to Duncan (1955) Multiple Range Test.

3. Results

3.1 Obesity assessment using the Body Mass Index (BMI)

The body mass index (BMI) of rats of the different groups was determined and the results are shown in Table (2).

It could be observed that feeding hypercholesterolemic diet raised the final body weight significantly compared to the basal diet. The average final body weight of the hypercholesterolemic control was 391.70 g while that of the normal control was 285.30g.

The calculation of the body mass index was done by dividing the final body weight in grams by the square of the final body length in cm.

The obtained results illustrated that the average gain in body weight at the end of the experimental period for normal control was 160.50g. The same table presents the result of the hypercholesterolemic control which showed that the average body weight gain was 265.67 g. So, there is a significant increase in body weight gain compared to the normal control.

On the other hand, gains of the weight in the 5, 10 and 20 g/L $MgSO_4$ treated groups were significantly lower when compared to the hypercholesterolemic group. They were 212.25, 201.42 and 174.10g respectively.

The same table shows that the body mass index (BMI) of the hypercholesterolemic control was significantly higher than that of the normal control. They were 0.72 and 0.52 g/cm^2 respectively.

The addition of $MgSO_4$ to the drinking water results in significant decrease in BMI of the magnesium treated groups relative to the hypercholesterolemic group.

It is obvious that the 20 g/L $MgSO_4$ treated group showed the lowest BMI value in the magnesium treated groups. Its value was very close to the value of normal group.

Table (2): Calculation of the body mass index (BMI):

Treatments	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Final body length (cm)	Body mass index (BMI) (g/cm^2)
Normal control (NC)	a 126.70 ± 1.09	c 285.30 ± 4.76	c 160.50 ± 4.12	a 23.60 ± 0.24	c 0.52 ± 0.01
Hypercholesterolemic control (HC)	a 124.33 ± 1.30	a 391.70 ± 6.35	a 265.67 ± 5.69	a 23.33 ± 0.21	a 0.72 ± 0.004
Treatment 1 (5 g /l $MgSO_4$)	a 126.08 ± 1.27	b 337.40 ± 0.88	b 212.25 ± 1.14	a 23.50 ± 0.22	b 0.61 ± 0.01
Treatment 2 (10 g /l $MgSO_4$)	a 125.83 ± 1.20	b 326.70 ± 1.73	b 201.42 ± 2.34	a 23.50 ± 0.22	b 0.59 ± 0.01
Treatment 3 (20 g /l $MgSO_4$)	a 124.40 ± 1.28	c 300.10 ± 3.61	c 174.10 ± 2.42	a 23.40 ± 0.24	c 0.55 ± 0.008
L.S.D	-	9.98	10.66	-	0.03

1) Values are shown as average ± standard error (n=6).
2) Different superscript letters in the same line indicate statistically significant difference for $P < 0.05$ (ANOVA).

3.2 Effect of Magnesium added to drinking water on Lipogram profile

Magnesium addition to the drinking water was associated with a significant reduction in plasma total cholesterol, triglycerides, LDL- cholesterol and VLDL- cholesterol levels while HDL- cholesterol was significantly increased, as judged from the pooled plasma samples in each group (Table 3).

Table (3): Lipogram profile for the different groups:

Treatments	Total cholesterol mg/dl	Triglycerides mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
Normal control (NC)	e 75.57 ± 3.53	d 79.13 ± 3.90	ab 53.00 ± 2.35	e 6.74 ± 0.78	d 15.82 ± 0.78
Hypercholesterolemic control (HC)	a 235.68 ± 12.9	a 158.61 ± 8.58	b 40.67 ± 3.40	a 163.29 ± 8.80	a 31.72 ± 1.72
Treatment 1 (5 g /l $MgSO_4$)	b 191.13 ± 4.79	b 122.30 ± 2.79	a 59.33 ±4.88	b 107.34 ± 7.10	b 24.46 ± 0.55
Treatment 2 (10 g /l $MgSO_4$)	c 154.26 ± 2.60	bc 107.05 ± 2.10	a 67.17 ± 3.40	c 65.69 ± 2.9	bc 21.41 ± 0.42
Treatment 3 (20 g /l $MgSO_4$)	d 132.62 ± 3.37	cd 93.80 ± 1.03	a 68.80 ± 2.65	d 45.06 ± 1.39	cd 18.76 ± 0.20
L.S.D	20.97	14.20	10.75	16.96	2.84

1) Values are shown as average ± standard error (n=6).
(2) Different superscript letters in the same line indicate statistically significant difference for $P < 0.05$ (ANOVA).

3.3 Effect of Magnesium added to drinking water on Liver function

The average activity of GOT, GPT and ALP in serum are shown in table (4) and they were about 69.00, 34.80 and 123.020 U/L for normal control while for hypercholesterolemic control were 107.17, 86.67 and 354.17 U/L

respectively. The results show significant increase in the values of GOT, GPT and ALP activities in hypercholesterolemic control relative to normal control. For $MgSO_4$ treated groups the values were significantly lower when compared to the hypercholesterolemic control.

Table (4): GOT, GPT and ALP activity for the different groups:

Treatments	GOT (U / I)	GPT (U / I)	Alkaline phosphatase (U / I)
Normal control (NC)	d 69.00 ± 3.69	e 34.80 ± 1.85	e 123.20 ± 7.55
Hypercholesterolemic control (HC)	a 107.17 ± 0.79	a 86.67 ± 4.20	a 354.17 ± 15.0
Treatment 1 (5 g /l $MgSO_4$)	ab 100.83 ± 2.18	b 69.67 ± 1.52	b 269.17 ± 9.95
Treatment 2 (10 g /l $MgSO_4$)	bc 92.50 ± 2.36	c 53.17 ± 2.83	c 217.33 ± 6.16
Treatment 3 (20 g /l $MgSO_4$)	c 88.20 ± 4.53	d 43.00 ± 0.70	d 175.00 ± 8.22
L.S.D	8.39	7.99	30.48

1) Values are shown as average ± standard error (n=6).
(2) Different superscript letters in the same line indicate statistically significant difference for $P < 0.05$ (ANOVA).

3.4 Effect of Magnesium added to drinking water on Serum magnesium

The data in Table (5) revealed that there was no change in serum magnesium of the normal and hypercholesterolemic control. They were 2.72 and 2.77 mg/dl respectively. This is because both groups were drinking tap water through the whole experimental period.

The values of serum magnesium in the magnesium treated groups increased significantly relative to the two control groups. The results were 3.75, 3.84 and 5.23 respectively. It was noticed that the maximum increase was in the 20 g/L $MgSO_4$ treated group.

3.5 Calculation of the exact dose of magnesium that each rat was consuming daily

Both of the normal and hypercholesterolemic controls were consuming about 0.0005 g Mg / 24 hour. The three magnesium treated groups was consuming more magnesium daily. The increase in magnesium consumption was significantly higher than that of the normal and hypercholesterolemic control.

Each rat in the 5, 10 and 20 g/L $MgSO_4$ treated groups was consuming about 0.025, 0.055 and 0.152 g Mg / 24 h respectively.

Table (5): Serum magnesium concentration of the different groups

Treatments	Magnesium (mg / dl)
Normal control (NC)	c 2.72 ± 0.14
Hypercholesterolemic control (HC)	c 2.77 ± 0.07
Treatment 1 (5 g /l $MgSO_4$)	b 3.75 ± 0.24
Treatment 2 (10 g /l $MgSO_4$)	b 3.84 ± 0.22
Treatment 3 (20 g /l $MgSO_4$)	a 5.23 ± 0.24
L.S.D	0.60

1) Values are shown as average ± standard error (n=6).
(2) Different superscript letters in the same line indicate statistically significant difference for $P < 0.05$ (ANOVA).

4. Discussion

The present study shows that magnesium addition to drinking water induces beneficial protective effects against

cardiac diseases in male albino rats fed high fat diet. The lipogram profile showed significant reductions in cholesterol, triglyceride, LDL- cholesterol and VLDL- cholesterol levels as well as significant increase in HDL-cholesterol in magnesium treated groups compared to the hypercholesterolemic group. The average total cholesterol of the hypercholesterolemic control was significantly higher than the normal control. Their values were 235.7 mg/dl and 75.6 mg/dl respectively. The total cholesterol of the magnesium treated groups 5, 10, 20 g/L $MgSO_4$ were significantly lower than that of the hypercholesterolemic control. They were 191.13, 154.26 and 132.6 mg/dl respectively.

It is clear that, the addition of $MgSO_4$ to the drinking water cause more resistance to the elevation of serum total cholesterol.

It was observed that the prevention of the elevation in the serum total cholesterol increase with the increase in $MgSO_4$ concentration in water.

As shown in Table (3), the average of triglycerides in the normal and hypercholesterolemic controls were 79.13 and 158.61 mg/dl respectively.

Serum triglycerides levels for the magnesium treated groups 5, 10, 20 g/L $MgSO_4$ were significantly lower than the hypercholesterolemic control. They were 122.30, 107.05 and 93.80 mg/dl respectively. It was noticed that the decrease in serum triglycerides level caused by the addition of 20 g/ L $MgSO_4$ was not significantly different than that caused by the addition of 10 g/L $MgSO_4$.

The elevation of serum LDL-Cholesterol is associated with increased risk of for coronary heart disease (CHD), it removes cholesterol from liver to other tissues.

The serum LDL-cholesterol in the normal control was 6.74 mg/dl while in the hypercholesterolemic control was 163.29 mg/ dl. The concentrations of the LDL-cholesterol for the other three groups 5, 10 and 20 g/L $MgSO_4$, were 107.34, 65.69 and 45.06 mg/dl respectively.

It was observed that the increase in $MgSO_4$ concentration in water caused significant resistance to the elevation in LDL-cholesterol levels relative to the hypercholesterolemic control.

HDL- cholesterol has a protective effect impeding plaque formation and shows an inverse relationship to chronic heart disease (CHD) prevalence. In fact, low HDL- cholesterol values constitute an independent CHD risk factor.

The data show that, the average HDL-cholesterol for normal control was 53.00 mg/dl but it was significantly lower in hypercholesterolemic control 40.67 mg/dl.

The consumption of $MgSO_4$ in drinking water caused significant elevation of the HDL- cholesterol levels relative to the hypercholesterolemic control.

The Values of the HDL- cholesterol in the 5, 10 and 20g/L MgSO4 treated groups were 59.33, 67.17 and 68.80 mg/dl, respectively. There was no significant difference in the increase in HDL- cholesterol levels among the three $MgSO_4$ treatments.

The average of serum VLDL-cholesterol for normal control was 15.82 mg/dl and was 31.72 mg/dl for hypercholesterolemic control.

The three $MgSO_4$ treated groups 5, 10 and 20g/L were significantly lower in serum VLDL-cholesterol relative to the hypercholesterolemic control. The VLDL-Cholesterol values of them were 24.46, 21.41 and 18.76 mg/dl respectively.

These results on the lipid profile are in agreement with other studies on the effect of magnesium on serum lipid fractions.

Altura *et al.,* 1990, emphasized that magnesium is highly effective in decreasing serum lipid fractions and in preventing the atherogenic process in rabbits. Also the study done by Olatunji and Soladoye 2007, who suggested that diet rich in magnesium could exert cardioprotective effect by reducing total cholesterol, triglycerides and ameliorated HDL-cholesterol, and Yaniv *et al.,* 1999, who confirm that magnesium fortification of drinking water is capable of inhibiting athrogenisis in male LDL-receptor deficient mice.

The liver function tests showed significant reductions in GOT, GPT and ALP levels in the magnesium treated groups which indicate the positive health impact of magnesium addition to water on liver. Elevated serum activity of these enzymes has been known to be indicators of risk of cardiovascular disease. The average activity of GOT, GPT and ALP in serum were about 69.00, 34.80 and 123.020 U/L for normal control while for hypercholesterolemic control were 107.17, 86.67 and 354.17 U/L respectively.

The results show significant increase in the values of GOT, GPT and ALP activities in hypercholesterolemic control relative to normal control.

For $MgSO_4$ treated groups the values were significantly lower when compared to the hypercholesterolemic control.

The results show that the GOT values of the 5, 10 and 20g/L $MgSO_4$ treated groups were 100.83, 92.50 and 88.20 U/L .There was only a slight difference between treatment 2 and 3.

The GPT values of the 5, 10 and 20 g/L MgSO4 treated groups were 69.67, 53.17 and 43.00 U/L respectively.

The values of the ALP activity for the 5, 10 and 20 g/L MgSO4 treated groups were 269.17, 217.33 and 175.00 U/L respectively.

All groups show a significant decrease when compared to the hypercholesterolemic control.

The body weight and body length were used to determine the Body mass index (BMI). There was a significant decrease in BMI of the magnesium treated groups relative to the hypercholesterolemic control.

The administration of magnesium in water also led to a significant increase in plasma magnesium concentrations. The values of serum magnesium in the magnesium treated groups increased significantly relative to the two control groups and reached its maximum in the 20 g/L $MgSO_4$ treated group.

Most of the results obtained in this study were dose dependent.

The mechanism by which magnesium affects heart diseases risks may include effects on enzymes and/ or hormones.

The results obtained from this study would provide information that could guide medical recommendations regarding the addition of Mg to water (Mg fortification of water) or bottled beverages.

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