ORIGINAL PAPER

THE EFFECT OF DIFFERENT FORMS OF FOOD DEPRIVATION ON CALCIUM AND MAGNESIUM CONCENTRATIONS IN THE SERUM, BRAIN AND FEMORAL BONE OF FEMALE WISTAR RATS*

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ABSTRACT

Hypocalcaemia and hypomagnesaemia in patients with eating disorders are poorly identified, same as in animal models of these diseases. The purpose of the present study was to assess the concentration of calcium and magnesium in the serum, brain tissue and femoral bone of food restricted female Wistar rats. Material and Methods: 48 rats (8-weeks old, 199 ± 18 g) were divided into 6 groups (n = 8): the control group (C) were fed *ad libitum*, with measurements of daily intake, as a baseline for the amount of intake calculation for the other groups. The remaining five were testing groups: R50 – received half a portion of the diet eaten by C. The other groups were fed with a 100% of the diet eaten by C, but in a different model of food restriction: RI-IV – one-four day/s feeding, followed by one-four day/s starvation, throughout the eight weeks of the experiment. After wet mineralization of tissues, the calcium and magnesium concentrations were measured via the AAS method. Results: The calcium concentrations in serum and brain were unchanged, while the concentration of calcium in bone samples was significantly higher in food deprived rats compared to control groups rats. The influence of different models of food deprivation was observed in magnesium concentrations in the tissues studied. Generally, the levels of this mineral were significantly lower in all tissues in rats exposed to starvation than in control. Conclusions: The results of the current study suggest that although the amount of food intake was similar in all starvation models, food deprivation affects the management of calcium and magnesium in different ways. The competitiveness between these

minerals, under food restriction, probably works to the benefit of calcium. This may lead to increased hypomagnesaemia and its subsequent implications.

Keywords: calcium, magnesium, brain, serum, femoral bone, animal anorexia model, female rats.

INTRODUCTION

Although anorexia nervosa was already described and diagnosed in the 19th century, its aetiology generally remains unclear. Anorexia nervosa is a severe pathology of eating behaviours characterized by weight loss which leads to a body mass of less than 85% of the expected weight (HADIGAN et al. 2000, CORCOS et al. 2001, MEHLER, MACKENZIE 2009). The body mass loss as a result of extremely low food intake usually corresponds with periods of starvation. Conversely, free will starvation is also used by healthy women as a simple method to lose weight and fight overweight and obesity. Our previous study (WOJCIAK, BUSIEL 2006) indicates that more than 70% of healthy women are not satisfied with their body shape and 50% of them have used, and continue to use, starvation as a method to control their body mass. This technique in recommended by many popular magazines as not only a healthy and easy method of weight control but also as way to "cleanse" the body. The scientific evidence for such a proven beneficial effect is rather poor and controversial. Data from research on anorexia have shown that low body mass in anorexics, as a result of extreme undernourishment because of low food intake and hunger, is responsible for numerous physiological and psychological dysfunctions and in some cases death (HADIGAN et al. 2000, MEHLER, MACKENZIE 2009). There are no data concerning healthy women using starvation periodically, especially in the context of measuring mineral concentrations.

This notwithstanding, magnesium and calcium deficiencies have been observed in patients with anorexia, in women who follow alternative, low-calorie diets as well as use starvation as a method of weight loss has been not presented often (MEHLER, MACKENZIE 2009, MISRA, KLIBANSKI 2011). A similar situation has been observed in animal studies, where the physiological significance of food restrictions or calorie restrictions is usually used to explain muscle dystrophy, growth and developmental problems rather than dysfunctions in micro-scales as the influence of starvation on vitamin and mineral metabolism (MATSUZAKI et al. 2005, HILLEBRAND et al. 2007, WOJCIAK 2013a,b, 2014a).

There are no data about the influence of either short or prolonged starvation on calcium and magnesium concentrations in rat tissues. Calcium and magnesium are those minerals which play very important roles in macro- and micro-scales of physiological processes. They are responsible for anything from bone structure to enzyme, hormone and neurotransmitter activation

and secretion as well as the functioning of the nervous system. The main dysfunctions reported by many authors in anorexia patients, as probably resulting from an insufficient calcium and magnesium intake, are: osteoporosis, osteomalatia and low bone mineral density as well as low mood, fatigue, impaired cognitive functioning and depression.

Animal models may help to advance our knowledge of anorexia, especially in explaining the influence of hunger on physiology (SIEGFRIED et al. 2003, MATSUZAKI et al. 2005). The rat food deprivation anorexia model, which consists of scheduled feeding and starvation periods, mimics the food restriction of anorexia patients. In our earlier studies on anorexia rat models we observed that different models of food restriction significantly disturb the zinc, copper, and iron metabolism, which also influences animals' behaviour (WOJCIAK 2013a,b, 2014a). In this study, we focused on the influence of different models of food restriction on calcium and magnesium distribution in the serum, brain and femoral bone of female rats.

EXPERIMENTAL PROCEDURE

Animals

Forty-eight female Wistar rats aged 8 weeks, with an intial body mass of 199 ± 18 g, were obtained from the Licensed Animal Facility in Brwinów, Poland. The rats used in this experiment were housed in a thermostatically controlled room (22°C ± 2) on a 12 h light/dark cycle (lights on at 8:00), humidity (55-65%) with free access to distilled water (*ad libitum*). All the animals were kept in individual stainless steel cages with neutral-plastic enamel. Animal care and handling for the experimental study was approved by the Local Regulatory Committee for animal studies (Approval No 37/05).

Food restricted model

The experimental procedure of food deprivation in animals has been partially described before as an anorexia model by SIEGFRIEDET al. (2003) and was modified for this study. At the beginning of the study, after a 3-day adaptation period, the animals were divided into 6 groups: control (C) and five testing groups (R50, RI, RII, RIII, RIV). The C group was fed *ad libitum*. The diet intake of this group was controlled every day, and was a base used to calculate the value of the diet given to the rest of the animals. R50 received daily half a portion of the diet consumed by C (chronic starvation model). The remainder of the groups received 100% of the diet eaten by C, but in a different model of food restriction (acute starvation model): RI – one day on, one day starvation; RII- two days on, two days starvation; RIII – three days on, three days starvation; and RIV – four days on, four days star-

vation. As a result, all test groups ate half of the diet normally consumed by the control group. All groups of rats were fed a standard, certified commercial diet for rodents Labofeed B (Morawski, Kcynia, Poland) for 8 weeks. The chemical composition of the diet was analytically assessed and was as follows: protein (19.9 ± 0.3%), carbohydrates (69.9 ± 0.4%), fat (2.7 ± 0.05%), dry mass (88.4 ± 0.1%), ash (7.5 ± 0.2%), calcium (1.49 ± 0.09%), magnesium (0.22 ± 0.02%).

At the end of the experimental period, all animals were anaesthetized by thiopental (40 mg kg^{-1} b.m.) intra-peritoneal injection, then dissected to collect tissues. The tissue samples were stored at -78°C. Blood samples were collected directly from the heart to PP tubes, and serum was obtained from blood samples by centrifugation at 5000 rpm for 10 minutes in room temperature. The serum was then stored frozen at -78°C.

Calcium and magnesium assay

The tissue samples, after being defrosted, were dried to dry mass and then wet mineralized in a microwave system with nitric acid (HNO$_3$, supra pure, 65%, w/w, Merck, Germany). After mineralization, the samples were quantitatively transferred to PP vials, using deionized water. The calcium and magnesium concentrations in the mineralized tissue samples were determined by the atomic spectrometry method with a graphite furnace spectrometer (AAS-5, Zeiss). As control for the calcium and magnesium measurement, control materials (Pig Kidney – CRM Brussels, Human serum – Randox UK were used. The aqueous standard for samples were Ca(NO$_3$)$_2$ and Mg(NO$_3$)$_2$ (Merck, Germany). The recovery rate of Ca and Mg was: 97-102% and 94-103%, respectively.

Data Analysis and Statistic

Data was expressed as the arithmetic mean ± SD. Group differences were assessed using one-way ANOVA followed by the Fisher's Least Significant Differences method. Data was deemed significant when $p < 0.05$.

RESULTS

The results obtained in this study are presented in Tables 1 and 2. The baseline data for the rats used in the study are displayed in Table 1. At the beginning of the experiment, all animals had a similar body mass (199 ± 19 g). Significantly, the daily food intake was twice as high in the control group (C) (18.8 g/d/rat) as in the others (~9.5 g/d/rat). The food intake in rats, calculated on the days with a diet serving, did not differ between the control and starvation groups, and was twice as high as the one by rats who ate half levels of the daily food ration. The food restricted rats systematically lost

Table 1

General developmental and nutritional characteristics of the groups of rats
(mean ± SD for 8 rats)

Parameter	Experimental groups						ANOVA
	C	R50	RI	RII	RIII	RIV	(*F*, *p*)
Diet intake (g/day/rat)	18.8[b] 1.6	9.9[a] 0.9	9.9[a] 10.0	9.2[a] 9.5	9.3[a] 9.9	9.7[a] 9.8	8.82 *p* < 0.001
Diet intake during food serving days (g/day/rat)	18.8[b] 1.6	9.9[a] 0.9	19.7[b] 6.0	18.5[b] 2.7	19.5[b] 1.1	19.4[b] 1.1	30.2 *p* < 0.001
Body mass gain (g/56 days)	25.5[c] 9.2	-41.6[b] 12.3	-49.0[ab] 10.1	-43.3[b] 12.6	-53.0[a] 6.6	-44.0[ab] 8.7	61.1 *p* < 0.001
Body mass at the end of study (g)	220.4[b] 17.9	155.1[a] 7.4	149.2[a] 11.6	156.1[a] 11.9	148.1[a] 20.1	158.2[a] 10.9	27.4 *p* < 0.001
Organ mass at the end of study (g)							
Brain (g) % of the body	1.73 0.10 0.78	1.74 0.13 1.12	1.74 0.05 1.17	1.71 0.09 1.09	1.71 0.17 1.15	1.72 0.17 1.09	0.06 ns
Femoral bone (g) % of the body	1.28 0.05 0.58	1.20 0.08 0.77	1.20 0.04 0.80	1.24 0.14 0.79	1.19 0.16 0.80	1.21 0.15 0.76	0.60 ns

a,b,c… – significant differences between groups at *p* < 0.05, ns – not significant

weight when compared to their body mass at the beginning of the study (ca. -45 g, body mass: ca. 155.0 g). However, the body weight of the control group increased (+25.5 g, body mass: 220.4 g). The greatest body weight loss was noted in the group starved 3 days (-53.0 g), the lowest in R50 (41.6 g). However, while all the models of food deprivation used in this experiment had a significant effect on reducing the body mass of all animals, the food deprivation had no effected on the organ weights of the rats. The mean mass of brains and femoral bones did not change in rats exposed to food restrictions in comparison to control (~1.7 g and ~1.2 g, respectively). Proportionally, the organs' share in the total body mass was higher in starvation groups (~1.1 % in brain and ~0.8 % in femoral bone) than in the control group (~0.8% in brain and 0.6% in femoral bone).

Table 2 shows the concentrations of calcium and magnesium in the serum, brain and femoral bone of rats used in experiment. The experimental models of food restrictions used in this study had a significant influence only on the serum magnesium level (*p* < 0.05), however there were significant differences between the mean control group and food restricted groups in magnesium concentrations in studied tissues and calcium levels in femoral bones. Generally, the reducing effects of starvation on magnesium concentrations in tissues were observed, *albeit* with some exceptions. The mean level of serum magnesium was significantly higher in C (3.20 mg dL^{-1}) than in R50, RIII and RIV (2.89, 2.92, 2.75 mg dL^{-1}, respectively) and similar in RI

Table 2

Calcium and magnesium concentrations in tissues of rats (mean ± SD)

Parameter	Experimental groups ($\overline{x} \pm$ SD)						Anova (F, p)
	C	R50	RI	RII	RIII	RIV	
Serum Ca (μg dL^{-1})	9.25 1.92	8.98 2.00	9.60 1.75	9.85 1.65	8.99 1.08	9.05 1.15	0.9 ns
Brain Ca (mg g^{-1} d.m.)	628.9 78.2	622.3 98.1	678.7 67.2	652.1 78.9	619.2 69.6	622.9 79.4	0.9 ns
Femoral bone Ca (mg g^{-1} d.m.)	199.4^a 21.6	206.8^{ab} 17.98	229.8^b 41.8	222.0^b 20.2	217.0^b 26.4	228.5^b 29.2	1.3 ns
Serum Mg (mg dL^{-1})	3.20^b 0.89	2.89^a 2.01	3.05^{ab} 1.80	3.11^{ab} 0.99	2.92^a 0.97	2.75^a 1.00	3.0 $p < 0.05$
Brain Mg (μg g^{-1} d.m.)	514.2^b 40.9	407.2^a 111.0	419.2^a 79.4	360.3^a 96.8	413.2^a 142.8	410.8^a 112.5	1.6 ns
Femoral bone Mg (mg g^{-1} d.m.)	3.91^b 0.12	2.97^a 0.11	2.89^a 0.07	3.00^a 0.13	2.98^a 0.14	3.02^a 0.12	1.9 ns

a,b,c… – significant differences between groups at $p < 0.05$, ns – not significant, d.m. – dry mass

and RII (3.05 and 3.11 mg dL^{-1}, respectively). The mean magnesium concentrations in the brain and femoral bone were significantly lower in all starvation groups (~415.0 μg g^{-1} d.m. and ~3.00 mg g^{-1} d.m., respectively) than in C (514.2 μg g^{-1} d.m. 3.91 mg $^{-1}$g d.m., respectively). A different situation was observed for the magnesium concentration than for the mean calcium tissues concentrations. The food restriction significantly increased the calcium concentrations in RI-RIV (~222.0 mg g^{-1} d.m.) and there were no differences in R50 (206.8 mg g^{-1} d.m.) in comparison to C (199.4 mg g^{-1} d.m.). Food deprivation had no effect on mean calcium concentrations in serum and brain samples, which were similar in all groups of rats (~9.3 mg dL^{-1} and ~637.3 μg g^{-1} d.m.).

DISCUSSION

Magnesium (Mg) and calcium (Ca) are those macro-minerals which play very extensive and important functions in physiology, from the structural role in bone building to the regulation of different physiological processes in an organism (TATSUMI et al. 2011, YAMAMOTO et al. 2011, LOPEZ-SACA et al. 2013). Such a wide range of actions performed by these elements means that their deficits may be unfavourable to multi-systems in an organism. Approximately half of the total Mg in the body exists in bones and plays an important role in bone metabolism. Mg deficiency is one of the risk factors for osteoporosis. Moreover, Ca is also an essential mineral that plays a crucial part in the maintenance of bone mass and prevention of fractures. Low Ca

intake can induce an increase in bone resorption and a decrease in bone mineral density. In the opinion of many authors, osteoporosis and low bone mineral density are common in anorexia nervosa patients. The reason for this is seen in the generally low food consumption, and hence low Ca intake. On the other hand, Matsuzaki et al. (2005) are of the opinion that bone impairment is dependent not only on Ca intake with food, but also on proportions and relations between Ca and Mg, especially in the context of a severe deficiency in one of those minerals. These authors also suggested that high Ca intake had no preventive effect on the alteration of bone metabolism in Mg-deficiency. The results of this study seem to confirm the above observations. Although the body mass of all food restricted animals decreased by about 30% in comparison to the control group, the bone masses were unchanged. Moreover, the Ca concentrations in the femoral bone were higher in those rats who consumed half the food portion and Mg levels were significantly decreased. This may suggest that more Ca than Mg is saved in the condition of food deprivation in rats.

Similar observations were made in the case of other analyzed tissues. The Ca concentrations in the serum and brains of food deprived rats were unchanged even after acute starvation (3 and 4 days of starvation), while Mg concentrations were significantly lower in these tissues. Probably because of its role in Ca^{+2} channels, Ca plays a very important part in the physiology of neurotransmitters and nerve signal conduction. This could be the reason why its concentration in the brain is stable. Korf et al. (1983) in a classical experiment, measured the regional Ca levels in rat and mouse brains in different conditions. The authors observed alterations in the Ca concentration in different brain areas only after an application of acute stress conditions that damaged nervous tissue enough to influence Ca metabolism. A similar situation was observed in the this study in respect of the brain tissue and the serum. Despite the fact that the rats were exposed to long-term chronic and acute food deprivation, the Ca concentration in serum samples was similar in both the control and starvation groups. The reason for this most probably lies with the homeostasis of this mineral, which can benefit from the enormous supply of calcium found in bones. Furthermore, the regulation of Ca homeostasis in organisms is mediated by many hormones (Tatsumi et al. 2011, Lopez-Saca et al. 2013).

Although Mg homeostasis is mediated by similar physiological mechanisms as that of Ca, the fact that Mg was at significantly lower levels in tissues of rats exposed to food deprivations than in control group shows that this mineral is less competitive in comparison to Ca. Rattanatayaran et al. (2001) have reported strong competition between Ca and Mg in Mg deficient rats. In the heart muscle, Mg decreased and Ca increased, implicating an increased cardiac risk. Moreover, Mg deficient rats had a tendency towards anorexia and low behavioural activity. Hypomagnesaemia is responsible for many serious dysfunctions, including psychological issues such as low mood and depression and, on the other hand, cognitive dysfunctions. The observa-

tion of this psychological emotional deprivation may be associated with low Mg concentrations in the brain, which have been observed in chronic and acute starvation contexts. These results are associated with data obtained by WOJCIAK (2014*b*) in a study of healthy women starved one or two days a week for 6 weeks. Mg concentrations, but not Ca, were significantly lower (about 20%) in the serum of these women after the study periods, and this was associated with low mood and depression. In a previous anorexia animal study, with female Wistar rats exposed to similar starvation periods as those presented in this article, rats also exhibited low physical activity, which was probably associated with low mineral intake (WOJCIAK 2014*a*). The food deprivation rat model has also been used in research into stress and depression mechanisms (KORF et al. 1983, RAPPAPORT et al. 1987, WOJCIAK 2014*a*). However, why rats change their behaviour when under the influence of food restriction is still unclear.

Although many studies have reported the impairment of bone structure and dysfunctions in osteocyte metabolism in anorexia nervosa as probably effected by an insufficient Ca intake and hypocalcaemia, hypomagnesaemia in patients with eating disorders is poorly reported in animal models of these diseases. RAJ et al. (2012) have reported hypomagnesaemia in 16% of patients with anorexia (in a population study examining more than 500 participants). BIRMINGHAM et al. (2004) observed low serum Mg levels in 60% of anorexics during a refeeding program. These and other (WEISELBERG et al. 2011) authors associate Mg deficiency with weakness, constipation and arrhythmias.

The present study shows that the monitoring of Ca status together with Mg status in anorexia patients is very important because Mg seems to be more sensitive to alterations than Ca. As suggested by WEISELBERG et al. (2011), the major medical compilations in anorexia and other eating disorders are due to decreased food intake, which in turn leads to a hypometabolic state. While most complications are reversible with recovery, some, such as bone loss, may not be. These authors are also of the opinion that of particular concern during recovery is the possible induction of a refeeding syndrome, which occurs as a consequence of changing from a catabolic to anabolic state of the body, causing hypophosphataemia, hypocalcaemia and hypomagnesaemia, which can lead to delirium, coma and death.

To sum up, the results of the current study suggest that although the amount of food intake was similar in all starvation models, acute and chronic food deprivation affects the management of calcium and magnesium in different ways. The competitiveness between these minerals, under food restriction, probably works for the benefit of calcium. This may lead to increased hypomagnesaemia and its subsequent implications.

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