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Uptake, distribution and excretion of magnesium in *Oreochromis mossambicus:* dependence on magnesium in diet and water

Marcel J.C. Bijvelds[1,2], Gert Flik[1], Zvonimir I. Kolar[2] and Sjoerd E. Wendelaar Bonga[1]
[1]*Department of Animal Physiology, Faculty of Science, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands;* [2]*Department of Radiochemistry, Interfaculty Reactor Institute, Delft University of Technology, Mekelweg 15, 2629 JB Delft, The Netherlands*

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Abstract

The euryhaline Mozambique tilapia *(Oreochromis mossambicus)* shows a more marked ability to adapt to low magnesium levels in food and water than many other fish species. Nonetheless, the internal distribution of magnesium is altered under low-magnesium conditions. The amount of magnesium in scales, and to a lesser extent the vertebral bone, is reduced and hence serve as magnesium reservoirs. The magnesium concentration of muscle is only marginally reduced by low external magnesium, suggesting that magnesium is partitioned to ensure normal muscle functioning. The water magnesium is of vital importance, as exposure to low-magnesium water markedly deminished the ability of tilapia to adapt to low-magnesium feeding. However, magnesium intake from the water, either *via* the integument or drinking, does not increase in low-magnesium fed fish, despite an increased opercular chloride cell density. The growth related magnesium accumulation of tilapia under low-magnesium conditions approximates the total intake of the element (from the food and from the water), indicating that magnesium losses are minimized and that the magnesium absorption from the gastrointestinal tract may be highly efficient and very important.

Introduction

Several previous studies have indicated that freshwater teleosts primarily depend on dietary rather then water borne magnesium (Ogino and Chiou 1976; Ogino *et al.* 1978; Knox *et al.* 1981; Gatlin *et al.* 1982; Shim and Ng 1988; Dabrowska *et al.* 1989; Shearer 1989; Dabrowska *et al.* 1991; Reigh *et al.* 1991; Van der Velden *et al.* 1991c, 1992). From these studies it can be concluded that most freshwater fish maintain optimal growth and magnesium homeostasis when the magnesium content of the food is about 15 to 20 mmol kg^{-1} (Cowey *et al.* 1977; Ogino and Chiou 1976; Ogino *et al.* 1978; Gatlin *et al.* 1982; Reigh *et al.* 1991; Shim and Ng 1988). Diets of lower magnesium content may lead to a decrease in growth rate, magnesium depletion of tissues, loss of muscle tonus, sluggishness, loss of apetite and, eventually, high mortality.

Whilst the importance of dietary magnesium is widely recognized, the role of magnesium in the ambient water on magnesium homeostasis of fish has been addressed only sparingly. Most freshwater environments contain an inexhaustible magnesium source, and fish can transport magnesium *via* integumentary routes. For example, when rainbow trout and carp were fed a low-magnesium diet, the amount of accumulated magnesium surpassed the amount of magnesium obtained from the food (Shearer 1989; Dabrowska *et al.* 1991). Conversely, when carp were fed a normal diet, a net integumental *efflux* was reported (Van der Velden

Correspondence: M.J.C. Bijvelds, at the above address: Tel: +31 24 3652876; Fax: +31 24 3652714

et al. 1991b). These results suggest that in some species a low dietary magnesium intake can, at least to some extent and under particular conditions, be compensated for by a net integumental magnesium uptake.

However, these studies do not exclude the possibility that freshwater fish may also obtain magnesium from the water *via* drinking or ingestion of water during feeding. But in juvenile Mozambique tilapia, *Oreochromis mossambicus*, in the weight range of 1 to 9 g, and fed a low-magnesium diet, showed low integumental uptake of magnesium and this uptake was not affected by the diet (Van der Velden *et al.* 1991a). Nevertheless, this species shows the remarkable capacity to adapt to low-magnesium feeding. Indeed, fish weighing around 200 g were shown to survive prolonged exposure to low-magnesium feeding without any effect on tissue mineral composition (Van der Velden *et al.* 1991c). Under these conditions the fish grew (albeit at a reduced rate) whilst accumulating little magnesium. It was concluded that this species maintains its magnesium balance by reducing magnesium loss and by obtaining magnesium from the water via an intestinal route.

In this paper we address the role of intestinal and branchial routes in magnesium uptake in Mozambique tilapia. The effects of low-magnesium feeding on mineral composition of tissues, blood plasma and urine were studied. Because earlier diet studies (Ogino and Chiou 1976; Shim and Ng 1988; Reigh *et al.* 1991; Van der Velden *et al.* 1991c, 1992) were inconclusive concerning the role of water born magnesium in the maintenance of magnesium balance, we also monitored the magnesium accumulation in fish kept in magnesium deficient fresh water, as well as in fish kept in fresh water containing magnesium. Magnesium uptake from the water was assessed by measuring the integumental magnesium intake and drinking rates, using a radiotracer method (Hazon *et al.* 1989; Van der Velden *et al.* 1991b). The effects of low-magnesium on ion regulatory mechanisms of the gills were also studied.

Materials and methods

Experimental conditions

Mozambique tilapia, *Oreochromis mossambicus* (Peters), of both sexes were obtained from laboratory stock, reared in Nijmegen tapwater (0.2 mmol l^{-1} Mg^{2+}) at 26°C under a photoperiod of 12 h of light alternating with 12 h of darkness. From this stock 160 fish, weighing about 40 g, were selected. Fish were weighed and randomly divided into four groups of 40 fish each and kept in 100 l, all-glass aquaria. The tapwater was replaced by artificial fresh water, *i.e.*, demineralised water with 0.4 mmol l^{-1} NaCl, 0.1 mmol l^{-1} Na_2SO_4, 0.06 mmol l^{-1} KCl, 0.8 mmol l^{-1} $CaCl_2$ and 0.2 mmol l^{-1} $MgSO_4$. The fish density in the aquaria did not exceed 16 g l^{-1} and the pH of the water was kept at 6.5 by titration with a concentrated NaOH solution. The water was recirculated over nylon filters and replaced by constant infusion at a rate of 20 l day^{-1}. Initially, all groups were fed a control diet (Hope Farms, Woerden, The Netherlands), at a ration of 2% of the total body weight per day; this food contained 30 mmol magnesium per kg (determined on food digested in concentrated HNO_3 with Inductively Coupled Plasma Atomic Emission Spectrophotometry, ICP-AES; Plasma IL200, Thermo Electron, USA). After three weeks the artificial fresh water in two groups was gradually replaced by artificial fresh water without magnesium, containing 0.3 mmol l^{-1} Na_2SO_4, 0.06 mmol l^{-1} KCl and 0.8 mmol l^{-1} $CaCl_2$. The water was maintained at a pH of 6.5 by titration with concentrated NaOH. After 7 days of replacement the magnesium concentration of the water was below 0.005 mmol l^{-1} (determined with ICP-AES). For two groups of fish, one kept in artificial fresh water and one kept in low-magnesium artificial fresh water, the control diet was replaced by a low-magnesium diet containing 1.5 mmol magnesium per kg food (Hope Farms, Woerden, The Netherlands). The magnesium concentration of the water was monitored weekly with the ICP-AES technique. The actual magnesium concentrations measured were 0.19 ± 0.02 (mean ± SD, n = 8) and 0.003 ± 0.002 (n = 8) mmol l^{-1}, for artificial fresh water and magnesium deficient artificial fresh water, respectively.

Preparation of samples

At the start of the experiment and after 2, 4 and 8 weeks fish were weighed and samples of six fish per group were taken. Blood samples of approximately 0.5 ml were taken by puncture of the caudal vessels with a heparinized syringe. Blood plasma was quickly separated by centrifugation for 1 min at 9000 × g. Blood plasma was ultrafiltered by centrifugation on Ultrafee-MC filtration units (Millipore, Bedford, USA) with a 10 kD molecular cut-off at 4800 × g for 30 min at 4°C. Samples were stored at -20°C until analysis. Fish were stunned by a blow on the head and the left operculum was excised. From the lateral side of the body, along the lateral line, ten scales were sampled. The fish were quickfrozen in dry ice, the belly was cut open, and the frozen urinary bladder with its contents was removed. Frozen urine was collected and stored at -20°C until analysis. A small portion of the dorsal musculature was collected and vertebrae just behind the anal fin were excised and cooked in a microwave for 3 min, in order to facilitate the separation of bone from adherent muscle tissue. Scales, muscle tissue and vertebrae were weighed to the nearest 0.1 mg, lyophilized, and weighed again. The lyophilized tissue samples (weighing less than 100 mg) were digested in 250 µl of HNO_3 for 1 h at 60°C and left overnight at room temperature. The remainder of the fish was weighed and digested in concentrated HNO_3 (1 ml per gram wet weight) at 60°C for 2 h and consecutively at room temperature for 48 h. All samples of digested tissue were stored at -20°C.

Sample analysis

Opercular chloride cell density

Freshly dissected opercula were incubated in tapwater containing 20 µmol l^{-1} 2-(4-dimethylaminostryryl)-*N*-ethyl-pyridinium iode (DASPEI; ICN Biomedicals, Zoetermeer, The Netherlands) for 4 to 6 h at 5°C. Opercula were rinsed in tapwater and the density of fluorescent cells established at anatomically fixed spots as described by Foskett *et al.* (1981). Twentyfive sites in a single operculum were scored. Chloride cell density is expressed as the number of DASPEI positive cells per mm^2.

Magnesium concentration of tissue samples

Plasma, urine, tissue and whole body digest samples were diluted with distilled water. Magnesium concentrations were determined with ICP-AES.

Radiotracers

The radionuclide ^{28}Mg was produced by neutron irradiation of a ^{6}Li enriched Li-Mg alloy with thermal neutrons in the Interfaculty Reactor Institute nuclear reactor (neutron flux 7×10^{16} s^{-1} m^{-2}), followed by a radiochemical separation resulting in an aqueous solution of [^{28}Mg]-$MgCl_2$ and its decay product ^{28}Al (Kolar *et al.* 1991). The specific activity of the ^{28}Mg product was approximately 0.5 GBq mol^{-1}. [^{51}Cr]-Cr(III)EDTA complex in an aqueous 5 mmol l^{-1} EDTA solution was purchased from Du Pont NEN Products ('s Hertogenbosch, The Netherlands). The specific activity of [^{51}Cr]-Cr(III)EDTA complex was approximately 10 TBq mol^{-1}.

Determination of integumental magnesium intake

Fish kept under control conditions and fish fed a low-magnesium diet for 10 weeks were housed in opaque containing 1.2 l of artificial fresh water (0.2 mmol l^{-1} Mg^{2+}). The water was constantly aerated and replaced at a rate of one 1 h^{-1}. The temperature was kept at 26°C by immersion of the containers in a thermostated water bath. After 36 h, the inflow of water was discontinued and 500 ml of water in the containers was replaced by artificial fresh water in which $MgSO_4$ was substituted by an equimolar amount of [^{28}Mg]-$MgCl_2$. The water was well mixed by aeration. At the start of the experiment 0.5 ml of water was sampled in 20 ml polyethylene counting vials for radioactivity determinations. The $^{28}Mg^{2+}$ concentration in the water was 60 kBq l^{-1}. After a 2 h exposure, fish were anaesthetized by adding 2 g of 3-aminobenzoic acid ethyl ester (MS-222; Sigma, St Louis, USA) to the water. Fish were taken out of the containers and rinsed thoroughly in running tapwater for 1 min. A blood sample (0.5 ml) was taken by puncture of the caudal vessels with a heparinized syringe. After the fish was quick-frozen in dry ice, the belly was cut open and the frozen gastrointestinal tract was removed. The remainder of the fish was weighed and homogenized in a tissue

blender. A sample of fish homogenate was put into a 20 ml polyethylene counting vial and weighed. The filling height of the counting vials was made identical for all fish samples; to the water samples an equal amount of non-radioactive fish homogenate was added and stirred for 1 h, assuring that the counting geometry and self-absorption of γ-rays was identical for all samples. Radioactivity was determined by counting of the 31 keV γ-ray emission of ^{28}Mg in a well type 7.6 cm × 7.6 cm NaI(Tl)-scintillation detector (Type 12SW12, Harshaw Chemie, De Meern, The Netherlands) connected to a PC-based multichannel analyzer (PCA-II, The Nucleus, Oak Ridge, USA). The counting time was 2000 to 3000 s. Counting rates were corrected for background and radioactive decay. The magnesium intake rate from the water, F_{Mg} (in nmol h^{-1}), was calculated using the formula:

$$F_{Mg} = \frac{q_f}{q_w \cdot t} \times Q_w$$

where Q_w is the quantity of magnesium in the water compartment at the beginning of the experiment (in nmol), q_f is the tracer quantity in the fish *minus* the gastrointestinal tract after 2 h (in counts per second, cps), q_w is the tracer quantity in the water at the start of the experiment (in cps) and t is the time (in h). Note that here a time independent F_{Mg} is assumed for $t \leq 2$ h. Values are expressed per 100 g fish.

Determination of drinking rate

Drinking rate was determined by a modification of the method described by Hazon *et al.* (1989). Fish kept under control conditions and fish fed a low-magnesium diet for 10 weeks were housed in opaque containers as described for determination of magnesium intake. After 36 h, the inflow of water was discontinued and 2 MBq l^{-1} [^{51}Cr]-Cr(III)EDTA complex was added to the water. At the start of the experiment, 1 ml of water was sampled in 20 ml glass counting vials. After 3 h fish were anaesthetized, rinsed, weighed, and a blood sample was taken (see above). Whole blood samples were put into 20 ml glass counting vials. After the fish was quick-frozen, the belly was cut open and the frozen gastrointestinal tract was taken out and put into a 20 ml glass counting vial.The filling height of all counting vials was adjusted, obtaining a uniform counting geometry for all samples. Radioactivity was determined on the basis of the 320 keV γ-ray emission of ^{51}Cr. Samples were counted for 100 to 1000 s, using a set-up as described for determination of magnesium intake. Counting rates were corrected for background and radioactive decay. The water intake rate, F_w (in μl h^{-1}), was calculated according to:

$$F_w = V_s \frac{q_b}{q_s \cdot t}$$

where V_s is the volume of the water sample (in μl), q_b is the quantity of tracer in the belly content after 3 h (in cps), q_s is the quantity of tracer in the water sample (in cps) and t is the time (in h). Note that here a time independent F_w is assumed. Pilot experiments indicated that this assumption was justified for experiments lasting up to 4 h. Values are expressed per 100 g fish.

Calculation and statistical analysis

The size of the scale, muscle and bone compartments of Mozambique tilapia were calculated from total fish weight, according to the equations reported by Van der Velden *et al.* (1989). These equations describe the relationship between the fish weight and the weight of the scale, bone and soft tissue compartments; the size of the muscle compartment was estimated at 70% of the total weight of the soft tissue (Van der Velden *et al.* 1989). Magnesium content of tissues (in μmol) was calculated from the tissue magnesium concentration (in μmol g^{-1}) and the calculated weight of the body part (in g). Whole body magnesium content as a function of time was fitted to a linear model using a regression analysis computer programme (Statgraphics, Statistical Graphics Corporation, Rockville, USA).

Differences among groups were assessed by means of a one-way analysis of variance (ANOVA). Subsequently, significance of differences between mean values was tested with the Tukey multiple comparisons test. Differences in urine magnesium concentration were statistically

assessed using the Kruskal-Wallis non-parametric ANOVA. Statistical analysis of data on integumental magnesium intake and drinking rate was conducted using the two-tailed Student's *t*-test, after the data had been analyzed for normal distribution. Statistical significance was accepted at $p < 0.05$. Values are depicted as mean ± SEM.

Results

Growth rate

Table 1 lists the average weight of fish exposed to a low-magnesium diet (LD), low-magnesium water (LW), or both (LDLW), at the start of the experiment, and after 2, 4 and 8 weeks. The control group was kept in water containing 0.2 mmol l^{-1} Mg^{2+} and was fed a diet containing 30 mmol kg^{-1} magnesium. No significant weight differences were found between groups.

Table 1. Whole body wet weight (g) of Mozambique tilapia exposed to a low-magnesium diet (LD), low-magnesium water (LW), or both (LDLW). The control group was kept in water containing 0.2 mmol l^{-1} Mg^{2+} and was fed a diet containing 30 mmol kg^{-1} magnesium

	Time (weeks)			
	0	2	4	8
Control	41.3±1.9	47.2±2.2	57.4±3.6	62.3±4.3
LD	39.5±1.6	43.8±1.8	46.7±2.1	53.4±2.9
LW	38.1±1.6	42.1±1.8	48.4±2.1	59.5±2.8
LDLW	40.0±1.5	45.3±1.6	53.2±2.5	59.8±3.5

Data are shown as means ± SEM (n = 6).

Whole body and tissue magnesium concentration

In Table 2 the magnesium concentration of the whole body and of scales, muscle and vertebral bone of the respective experimental groups are summarized. Whole body magnesium concentrations and magnesium concentrations of scales, muscle and vertebral bone decreased in the LDLW group. A decreased magnesium concentration was first observed in the scales (week 2), after 4 weeks in the vertebral bone and the whole body, and after 8 weeks in the muscle tissue. From week 4 onwards, the magnesium concentration of the scales was also affected in the LD group. The magnesium concentrations of the whole body and tissues of the LW group remained at the control level, except for a slight decrease of the scale magnesium concentration after 8 weeks.

Table 2. Whole body magnesium concentration (a: µmol g^{-1} wet weight) and magnesium concentration in scales (b), muscle (c) and vertebral bone (d: µmol g^{-1} dry weight) of Mozambique tilapia exposed to a low-magnesium diet (LD), low-magnesium water (LW), or both (LDLW). The control group was kept in water containing 0.2 mmol l^{-1} Mg^{2+} and was fed a diet containing 30 mmol kg^{-1} magnesium

	Time (weeks)			
	0	2	4	8
	a: [Mg] of the whole body (µmol g^{-1} wet weight)			
Control	15.2±0.6	14.3±0.4	14.7±0.4	14.5±0.3
LD	14.7±0.4	14.7±0.3	14.3±0.3	14.6±0.3
LW	14.8±0.5	15.1±0.3	13.9±0.3	14.8±0.1
LDLW	14.9±0.4	13.7±0.3	12.3±0.6**	11.3±0.4***
	b: [Mg] of scales (µmol g^{-1} dry weight)			
Control	106.2±1.4	98.6±3.1	96.7±2.3	103.6±2.7
LD	104.0±2.4	95.5±3.1	83.5±2.8**	89.1±2.7***
LW	104.2±3.4	96.8±1.7	99.8±2.5	94.3±1.7*
LDLW	105.9±3.0	83.7±2.6**	75.2±2.2***	63.7±1.1***
	c: [Mg] of muscle tissue (µmol g^{-1} dry weight)			
Control	51.4±1.8	48.0±1.7	47.2±3.1	48.1±1.3
LD	48.8±1.5	50.5±1.2	49.3±1.4	48.2±1.1
LW	50.9±1.3	46.3±1.3	48.4±1.9	49.6±0.9
LDLW	51.0±2.0	46.4±1.1	46.8±2.0	43.2±1.4*
	d: [Mg] of vertebral bone (µmol g^{-1} dry weight)			
Control	119.6±4.8	118.8±4.9	115.9±2.4	120.6±4.9
LD	113.3±2.1	115.7±2.0	116.9±3.5	108.4±4.1
LW	119.8±4.7	117.5±6.0	114.5±2.8	117.3±2.4
LDLW	113.8±4.5	112.7±2.7	92.4±3.7***	81.7±4.9***

Data are shown as mean ± SEM (n = 6); values marked with an asterisk are significantly different from the control value in the same column; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Growth and magnesium in the whole fish and in muscle and scalar and vertebral bone

Figure 1a shows the growth-related changes in whole body magnesium content of Mozambique tilapia exposed to a low-magnesium diet (LD), low-magnesium water (LW) or both (LDLW), over a 8 week period, calculated from the mean fish weight (Table 1) and the whole body magnesium concentration (Table 2). Regression analysis of weighed data yielded average magnesium up-

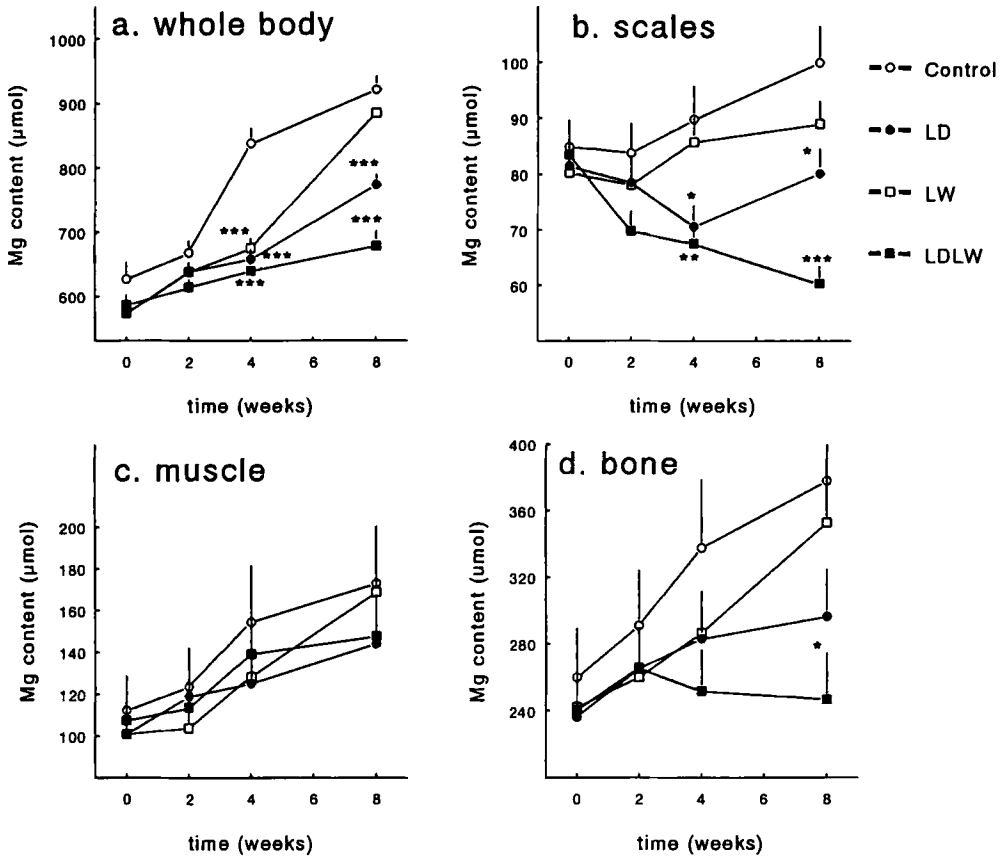

Fig. 1. Magnesium content (in µmol) of the whole body (1a), scales (1b), muscle tissue (1c) and vertebral bone (1d) of Mozambique tilapia fed a low-magnesium diet (LD), kept in low-magnesium water (LW) or both (LDLW). Magnesium content was calculated from the average body weight and the respective whole body or tissue magnesium concentration, as described in the Materials and methods section. In some cases the error bars are obscured by the symbols. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, compared to the control value at the same time.

take rates of 5.3 ± 1.3 (correlation coefficient, r, is 0.92, p = 0.052; n = 4), 3.4 ± 0.4 (r = 0.98, p = 0.012), 5.9 ± 0.6 (r = 0.99, p = 0.009) and 1.7 ± 0.1 (r = 0.99, p = 0.040) µmol day^{-1}, for the control group, LD group, LW group, and the LDLW group, respectively.

The magnesium content of tissues (*viz.*, the total amount of magnesium in the respective tissue compartments, expressed per fish) was calculated from the dry weight of the body part and the tissue magnesium concentration (see Table 2), as described in the Materials and methods section. The magnesium content of the scales of fish fed a low magnesium diet (LD and LDLW groups) was significantly lower than of the control group from week 4 onwards (Fig. 1b). The magnesium content of the scales of the LDLW group at 4 and at 8 weeks was also significantly lower than at the start of the experiment ($p < 0.01$, two-tailed Student's t-test), indicating that a net magnesium loss from the scales had occurred in this group. Figure 1c shows that the magnesium content of the muscle tissue was not affected. The magnesium content of the bone tissue of the LDLW group at 8 weeks was significantly lower than that of the control group (Fig. 1d).

Magnesium concentration in plasma and urine

The magnesium concentration in plasma ($[Mg_{pl}]$), ultrafiltered plasma ($[Mg_{pf}]$) and urine $[Mg_{ur}]$ at the start of the experiment and after 8 weeks are shown in Table 3. No significant changes were observed

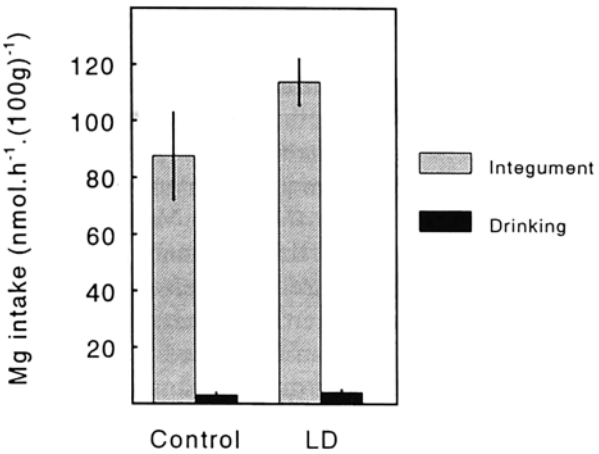

Fig. 2. Rate of magnesium intake *via* the integument (n = 6) and *via* drinking (n = 8) of Mozambique tilapia fed a control diet or a low-magnesium diet (LD), kept in fresh water containing 0.2 mmol l^{-1} magnesium. Magnesium intake *via* drinking was calculated from the drinking rates and the magnesium concentration of the water.

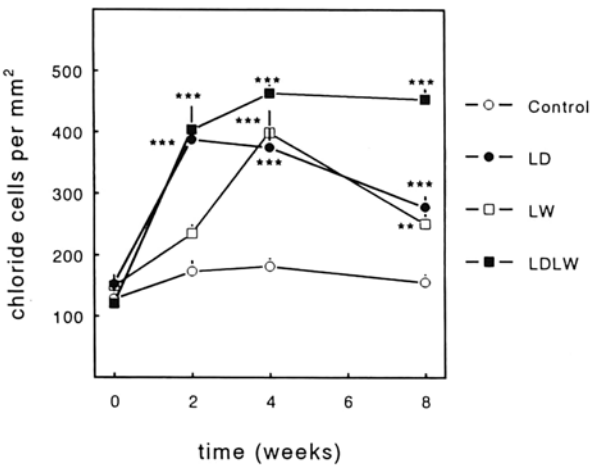

Fig. 3. Opercular chloride cell density of Mozambique tilapia fed a low-magnesium diet (LD), kept in low-magnesium water (LW) or both (LDLW). The control group was kept in water containing 0.2 mmol l^{-1} Mg^{2+} and was fed a diet containing 30 mmol kg^{-1} magnesium. ** $p < 0.01$; *** $p < 0.001$, compared to the control value at the same time (n = 6).

in the magnesium concentration of total plasma and plasma filtrate during the 8 week period of experimentation. Magnesium concentration of urine varied considerably between individual fish. For the control group the concentrations observed ranged from 0.47 to 1.96 mmol l^{-1}. At 0, 2 and 4 weeks, no significant differences were observed in urine magnesium concentration between groups. At 8 weeks, the urine magnesium concentration of the LDLW group was significantly lower then all other groups, and it was also lower then at the start of the experiment.

Magnesium intake from the water

Figure 2 shows the magnesium intake from the water *via* the integument and *via* drinking in fish fed a control diet and in fish fed a low-magnesium diet (LD). The integumental magnesium intake of the LD group was not significantly different from the control group: 113.8 ± 8.6 nmol h^{-1} $(100\ g)^{-1}$ (n = 6) and 87.6 ± 15.6 nmol h^{-1} $(100\ g)^{-1}$ (n = 6, p = 0.17), respectively. The magnesium intake *via* drinking was calculated from the drinking rates of 15.3 ± 5.9 µl h^{-1} $(100\ g)^{-1}$ (n = 8) and 20.3 ± 5.8 µl h^{-1} $(100\ g)^{-1}$ (n = 8) for the control group and the LD group respectively, and the magnesium concentration of the water (0.2 mmol l^{-1}).

Opercular chloride cell density

Figure 3 shows the opercular chloride cell density during the course of the experiment. The opercular chloride cell density increased significantly within 2 weeks after the start of the experiment in fish fed a low-magnesium diet (LD and LDLW groups). Fish kept in low-magnesium water had an increased opercular chloride cell density after 4 weeks of exposure, whereas the chloride cell density of the control group remained constant throughout the experiment. After 8 weeks, the LD and LW groups showed some recovery, but the chloride cell densities were still above control level. In the LDLW group, the opercular chloride cell density remained high.

Discussion

The role of magnesium in food and water in the magnesium homeostasis of a teleost fish, *Oreochromis mossambicus*, was investigated. Fish growing under magnesium-restricted conditions

for 8 weeks can maintain a net magnesium uptake; under these conditions, the amount of magnesium deposited in the body approximated the intake and magnesium loss was negligible. Magnesium intake from the water *via* the integument (gills) or *via* drinking was not enhanced by low-magnesium feeding. These facts indicate that intestinal absorption is highly efficient. The scales may serve as magnesium reservoir under magnesium-restricted conditions as the scale magnesium pool had significantly decreased over an 8 week period. A growth-related increase of the muscle magnesium pool was maintained, however, suggesting that magnesium is partitioned to ensure normal muscle functioning. The changes in the make-up of the branchial epithelium and the decrease in the urine magnesium concentration suggest that magnesium-restricted conditions trigger an ionoregulatory response.

Growth rate

Our data on Mozambique tilapia showed that growth rate was not affected by 8 weeks of low-magnesium feeding and this is in accord with previous observations on *Oreochromis aureus* (Reigh *et al.* 1991) and *Oreochromis niloticus* (Dabrowska *et al.* 1989). Conversely, our results differ from similar studies on carp, *Cyprinus carpio* (Ogino and Chiou 1976; Shearer 1989; Dabrowska *et al.* 1991; Van der Velden *et al.* 1992), rainbow trout, *Oncorhynchus mykiss* (Ogino *et al.* 1978; Knox *et al.* 1981), guppy, *Poecilia reticulata* (Shim and Ng 1988) and channel catfish, *Ictalurus punctatus* (Gatlin *et al.* 1982), in which a decrease in growth rate was observed during low-magnesium feeding. This discrepancy indicates a greater capacity of tilapia to adapt to magnesium-restricted conditions. However, when low-magnesium feeding of *O. mossambicus* lasted up to 14 weeks a decrease in body weight was observed (Van der Velden *et al.* 1991c). We conclude, therefore, that the absence of significant effects of low-magnesium feeding on growth rate resulted from the relatively short time course (8 weeks) of the experiment and that Mozambique tilapia fed a low-magnesium diet can maintain normal growth rates for at least 8 weeks.

Tissue magnesium distribution

A previous study on Mozambique tilapia weighing about 200 g showed that this species can adapt to a low-magnesium diet, enabling it to maintain normal magnesium concentrations in muscle tissue, scales and bone tissue (Van der Velden *et al.* 1991c). We show here that Mozambique tilapia, weighing about 50 g, are more sensitive to low-magnesium diets, in particular when the water magnesium concentration is reduced. Exposure to both a low-magnesium diet and low-magnesium water caused a decrease in the magnesium concentration of scales, vertebral bone and, eventually, also muscle. In fish fed a low-magnesium diet but kept in normal fresh water the decrease in magnesium concentration of the scales was less pronounced and magnesium concentration in muscle and bone tissue were not affected during the 8 weeks of experimentation. Concurrently, whole body magnesium concentration decreased only after simultaneous exposure to low-magnesium feeding and magnesium deficient water.

The size of the magnesium pool in scales, muscle and bone tissue (see Fig. 1b–d) was calculated from the tissue magnesium concentration and the size of the tissue compartment, which is a function of fish weight (Van der Velden *et al.* 1989). For the LDLW group, it can thus be shown that the quantity of magnesium deposited in the scales decreased from 83.5 ± 4.4 μmol per fish at the start of the experiment to 60.3 ± 3.1 μmol per fish ($p < 0.01$, two-tailed Student's *t*-test) after 8 weeks. This indicates that under severe magnesium-restricted conditions magnesium can be withdrawn from the scales. For bone tissue, the corresponding values are 240.4 ± 22.8 μmol per fish and 246.8 ± 27.7 μmol per fish, respectively, indicating that virtually no magnesium was accumulated in the bone compartment, even though fish grew and new bone tissue was formed. It seems plausible that bone magnesium, like magnesium in scales, is redistributed, foremost within the bone compartment itself as it seems unlikely that newly formed bone tissue was completely devoid of magnesium. It is clear then that the distribution of magnesium in the body is altered when the uptake of magnesium is low; the scale magnesium pool, and possibly the bone magnesium pool, are mobilized for maintaining normal magnesium concentrations in other

parts of the body, *e.g.*, in the muscle tissue where the growth related increased of the magnesium pool was not affected. Magnesium is crucial for muscle function, and therefore the ability of Mozambique tilapia to maintain a normal magnesium concentration in the muscle tissue under conditions of restricted magnesium availability reflects an important regulatory mechanism. Previously, it was observed that demineralisation of scalar and vertebral bone occurred in response to low-calcium stress in this species (Flik *et al.* 1986). It suggests that these hard tissue compartments function as mineral reservoirs under deficiency conditions.

Except for a slight decrease in the magnesium concentration of the scales, exposure to low-magnesium water alone had no effect on tissue or whole body magnesium concentrations. But the opercular chloride cell density was increased, suggesting that a regulatory mechanism or stress response is induced by low ambient magnesium concentrations. This is in agreement with an earlier study which showed that the external magnesium concentration is inversely related to the osmotic water permeability of the gills (Wendelaar Bonga *et al.* 1983).

Magnesium uptake

From an integumental ^{28}Mg intake of 87.6 ± 15.6 nmol h^{-1} (100 g)$^{-1}$ we calculate an integumental magnesium intake of approximately 1 μmol day^{-1} for a 50 g Mozambique tilapia kept in fresh water containing 0.2 mmol l^{-1} magnesium. This value is in good agreement with an earlier study done in this laboratory (Van der Velden *et al.* 1991a), in which we reported on the integumental magnesium intake of juvenile Mozambique tilapia. Our present data indicate that the integumental magnesium intake rate did not increase significantly in fish fed a low-magnesium diet. Nevertheless, direct intake from the water is of particular significance when the dietary magnesium uptake is low: for fish fed a low-magnesium diet, the growth related magnesium uptake rate of 3.4 ± 0.4 μmol day^{-1} can only be explained by assuming that net magnesium intake via the integument occurs. The role of the water as a magnesium source is further indicated by the observation that reduction of the water magnesium concentration aggravates the symptoms of low-magnesium feeding and, concurrently, reduces the growth related magnesium uptake.

Drinking rates were determined on the basis of the accumulation of [^{51}Cr]-Cr(III)EDTA complex in the gastrointestinal tract (Hazon *et al.* 1989). No ^{51}Cr could be detected in blood samples, indicating that the amount of [^{51}Cr]-Cr(III)EDTA complex absorbed from the gastrointestinal tract was negligible. In the experimental group fed a low-magnesium diet, drinking rates were maintained at the control level. From this drinking rate one can calculate that the uptake of magnesium via ingestion of water, even if one assumes that this magnesium load is completely absorbed, is negligible (approximately 0.04 μmol day^{-1} for a 50 g fish or less than 1% of the magnesium accumulated per day). These data lead us to conclude that a low dietary magnesium uptake is not compensated for by increment of the magnesium uptake from the water and that net magnesium accumulation under deficient conditions must, therefore, be secured by restricting magnesium losses via integumental and excretory routes.

Magnesium excretion

Euryhaline species govern renal electrolyte excretion both by control of the glomerular filtration rate and by control of reabsorption in the renal tubules and in the urinary bladder (Hickman 1968b; Hickman and Trump 1969; Loretz and Bern 1980; Oikari and Rankin 1985; Elger *et al.* 1987; Curtis and Wood 1992). Evidence for regulation of renal magnesium excretion comes from the observation that the magnesium concentration in bladder urine of fish fed a low-magnesium diet and kept in magnesium deficient water was significantly lowered after 8 weeks of exposure. This effect was not accompanied by a decrease in plasma magnesium concentration. Urine calcium concentration did not significantly change (1.57 ± 0.32 mmol l^{-1} at the start of the experiment *vs.* 1.24 ± 0.14 mmol l^{-1} after 8 weeks; $p = 0.52$, Mann-Whitney U-test), indicating that the lowered magnesium concentration can not be attributed to the production of a more copious, diluted urine. Moreover, we found no indications that would predict an enhanced water excretion: the drinking rates were at control level and the activity of the principal hormone involved in

Table 3. Magnesium concentration of plasma ($[Mg_{pl}]$), ultrafiltered plasma ($[Mg_{pf}]$) and urine ($[Mg_{ur}]$) of Mozambique tilapia exposed to a low-magnesium diet (LD), low-magnesium water (LW), or both (LDLW). The control group was kept in water containing 0.2 mmol l^{-1} Mg^{2+} and was fed a diet containing 30 mmol kg^{-1} magnesium

	Time (weeks)					
	0			8		
	$[Mg_{pl}]$	$[Mg_{pf}]$	$[Mg_{ur}]$	$[Mg_{pl}]$	$[Mg_{pf}]$	$[Mg_{ur}]$
Control	1.16±0.07	0.76±0.07	1.30±0.25	0.99±0.12	0.65±0.09	0.87±0.08
LD	1.09±0.08	0.79±0.06	1.12±0.26	0.86±0.13	0.70±0.09	1.10±0.37
LW	1.06±0.07	0.78±0.04	1.28±0.30	0.97±0.15	0.69±0.10	0.94±0.31
LDLW	0.99±0.06	0.74±0.04	0.98±0.27	0.88±0.15	0.62±0.11	0.44±0.06*

Data are shown as mean ± SEM (n = 6); values marked with an asterisk are significantly different from the control value in the same column. *$p < 0.05$.

epithelial water permeability, prolactin (Wendelaar Bonga 1993), was unaffected (as judged from morphometric analysis of the prolactin cells of the pituitary gland; unpublished results). Magnesium excretion may principally be regulated by reabsorption in the kidneys and the bladder. Accordingly, the observed large spread in urine magnesium concentrations of the control group may be inherent to sampling procedures and result from reabsorption of magnesium during storage of urine in the bladder. From an average urine flow of 40 ml day^{-1} kg^{-1} fish (Hickman and Trump 1969), we calculate that the magnesium excretion in a 50 g Mozambique tilapia decreases from 2 μmol day^{-1} under control conditions to 0.9 μmol day^{-1} for fish fed a low-magnesium diet and kept in low-magnesium water.

Besides the renal pathway, magnesium may also be lost via the integument and via the gastrointestinal tract. Data of direct determinations of *extra*renal magnesium efflux in tilapia are to our knowledge not available. This is in our opinion mainly due to the fact that a suitable magnesium tracer, which must essentially be carrier-free for determination of integumental efflux under steady-state conditions, has not been used. Oikari and Rankin (1985) showed that in freshwater rainbow trout, even after intraperitoneal infusion of a large magnesium dose, the *extra*renal net magnesium efflux was below the detection level of 1 μmol kg^{-1} h^{-1}, and less than 1% of the amount excreted renally. Also, for marine southern flounder *(Paralichthys lethostigma)* it was reported that excretion of absorbed magnesium occurs exclusively *via* the kidneys (Hickman 1968a). Under normal dietary conditions the net accumulation rate for magnesium of a 50 g fish (5.3 ± 1.3 μmol day^{-1}) amounts to 18% of the dietary intake (30 μmol day^{-1}). Because renal magnesium excretion cannot account for this relatively large apparent magnesium loss (30 – 5.3 = 24.7 μmol day^{-1}), we suggest that only a minor part of the magnesium load is actually absorbed from the gut, as was reported for the marine southern flounder (Hickman 1968a), or that magnesium absorbed is secreted in more distal parts of the gastrointestinal tract. However, under conditions of restricted magnesium feeding the magnesium deposition in the body is balanced by the intake (from the food and from the water) minus the renal excretion. This implies that under these conditions a) magnesium absorption in the intestinal tract is highly efficient, and that b) the flow of magnesium to the water from the fish is very low. We here show that an increase of the opercular chloride cell density was induced by both exposure to Mg^{2+} deficient water and low-magnesium diet. The increase was comparable to that induced by exposure of Mozambique tilapia to acidified water (Wendelaar Bonga *et al.* 1990), and was more pronounced than the increases induced by exposure to a low ambient calcium concentration (Pratap and Wendelaar Bonga 1993), or treatments with prolactin (Flik *et al.* 1994) or growth hormone (Flik *et al.* 1993). The high chloride cell density seen under magnesium-restricted conditions suggests that enhanced branchial influx of ions is required to compensate for losses of ions due to changes in the (magnesium-dependent?) permeability of the branchial epithelium. However, we found no evidence for enhanced prolactin

activity that could have indicated changes in branchial permeability. We therefore suggest that Mozambique tilapia can maintain a low branchial permeability to magnesium, without activating its prolactin cells, thus restricting magnesium losses. A search for an integumental (branchial) cellular component in magnesium uptake from the water seem warranted.

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