Role of Lipid Peroxidation and Vitamin E in Magnesium Deficiency

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Zusammenfassung

Es wurde untersucht, ob Lipidperoxidation an den Erscheinungen des Mg-Mangels beteiligt ist. Dazu wurde 115 g schweren männlichen Wistarratten eine Kontroll-, eine Mg-arme, eine Mg-arme plus Vitamin E-arme und eine Mg-arme Vitamin E-reiche Diät 11 Wochen lang verfüttert. Anschließend wurden Vitamin E, Malondialdehyd und der Mineralgehalt in Serum, Leber, Herz, Nieren, Aorta, Hoden und Skelettmuskel gemessen.
Mg Mangel verminderte den Vitamin E-Gehalt im Serum und allen untersuchten Geweben. Vitamin E-Supplementation erhöhte den α-Tocopherolgehalt besonders in der Leber. Mg-Mangel erhöhte den Malondialdehyd-Gehalt, besonders bei Vitamin E-reduzierter Diät. Die durch Mg-Mangel hervorgerufenen Veränderungen im Mineralgehalt der Gewebe, die Erytheme, die malignen T-Zellymphome, Leukämien und Tumore wurden durch Vitamin E-Supplementation nicht verhindert. Nur die durch Mg-Mangel entstandenen Hautulzerationen wurden durch Vitamin E signifikant vermindert.

Summary

The aim of the study was to ascertain whether lipid peroxidation is involved in the effects of Mg deficiency. Male Wistar rats, weighing 115 g, were fed a control, an Mg-deficient, an Mg-deficient plus vitamin E-reduced and an Mg-deficient-vitamin E-supplemented diet for 11 weeks. At the end of the experiment, vitamin E, malondialdehyde and mineral content were measured in serum, liver, heart, kidney, aorta, testis and skeletal muscle. Mg deficiency reduced vitamin E in serum and all examined tissues. Vitamin E supplementation of Mg-deficient rats produced only a slight increase in α-tocopherol levels, except for the liver, which stored a considerable amount.
Mg deficiency increased malondialdehyde, particularly in rats also fed the low vitamin E diet. Mg deficiency-induced alterations in mineral content of the tissues, erythema, development of malignant T cell lymphoma, leukemia and development of tumors were not prevented by vitamin E supplementation. Only skin ulcerations in Mg deficiency were significantly reduced by vitamin E.

Résumé

Le but de la présente étude était de vérifier l'éventuelle implication de la péroxydation des lipides dans les effets des déficits en magnésium. Des rats Wistar mâles pesant 115 g ont reçu pendant 11 semaines les différents régimes alimentaires suivants: régime témoin, régime carencé en Mg, régime carencé en Mg et pauvre en vitamine E et, enfin, régime carencé en Mg et supplémenté en vitamine E. A la fin de l'expérimentation, les auteurs ont mesuré les taux sériques, hépatiques, cardiaques, rénaux, aortiques, testiculaires et musculosquelettiques de vitamine E, d'aldéhyde malonique et de substances minérales.
Le déficit magnésique a réduit les concentrations de vitamine E dans le sérum et dans tous les tissus examinés. Une supplémentation en vitamine E chez les animaux carencés en Mg a entraîné une augmentation des taux d'α-tocophérol, en particulier dans le foie. Les altérations de la teneur tissulaire en substances minérales, les érythèmes, les lymphomes des cellules T, les leucémies et les effets tumorigènes induits par la carence magnésique n'ont pas été minimisés par la supplémentation en vitamine E, qui n'a réduit significativement que les ulcérations cutanées provoquées par le déficit en Mg.

Introduction

Significant effects of experimental Mg deficiency in rats are transient erythema and edema, skin lesions, reduced growth, development of malignant T-cell lymphoma and leukemia, development of intestinal tumors, calcification of kidneys and large blood vessels, changed intracellular mineral content, cardiac necroses and increased collagen content in heart and neuromuscular hyperexcitability [1,2, 3, 4, 5].
The basic pathobiochemical mechanism of Mg deficiency may be an alteration of the Mg-Ca-antagonism in the extracellular fluid and at membranes due to reduced extracellular Mg^{2+} concentration [6]. This effect is an explanation for the Mg deficiency-increased cell membrane permeability and ion turnover and the increased effect of hormones on smooth muscle cells acting via Ca^{2+} influx and rise in $[Ca^{2+}]_i$. The altered Mg-Ca ratios may also lead to the increased Ca-dependent release of some hormones, such as catecholamines, prostaglandins and related substances [6].
Moreover, in Mg deficiency, increased lipid peroxidation (LPO) was found in isolated liver mitochondria [7] and in liver in vivo [8]. Mg deficiency-induced cardiac necrosis was effectively reduced in a dose-dependent manner by simultaneous administration of vitamin E to Mg deficient Syrian hamsters [9]. Since vitamin E is one of the most effective scavengers of free radicals [10], these results suggest that free radical mechanisms are

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involved in Mg deficiency-induced myocardial injury.
The same relationship may hold for human ischemic heart disease (IHD). Epidemiological studies revealed a strong inverse correlation between IHD mortality and the serum level of vitamin E, whereas the classic risk factors cholesterol and diastolic blood pressure had only a moderate association with IHD [11]. Alteration of ion permeability and LPO, adversely affected by Mg deficiency, may act in accord. An increase in intracellular Ca^{2+} can enhance LPO [12]. Mg deficiency-increased release of catecholamines may increase LPO [13] and Mg deficiency-induced alteration of fatty acids in phospholipids may increase LPO. Increased LPO may lead to an increase in membrane permeability [14] and may enhance the effects of Mg deficiency. Since LPO may be a general pathological mechanism [14], LPO may play a significant role in the pathology of Mg deficiency. To clarify the realtionship between Mg deficiency and LPO, we fed a low vitamin E diet, and a vitamin E supplemented diet to rats on a Mg deficient diet.

Materials and Methods

After obtaining approval of local authorities and the Animal Protection Committee, the experiment with 175 male Wistar rats, weighing 115 g (Interfauna, Tuttlingen, FRG) was undertaken. Males were used, because of their greater susceptibility to free-radical-induced hepatotoxicity [15]. A control diet (described elsewhere [16]) was fed to 25 rats (group A); Mg-deficient diet was fed to 150 rats, devided into 3 groups: group B (Mg deficiency alone), group C (also vitamin E reduced), and group D (fed excess vitamin E supplements). Omission of Mg and vitamin E from the control diet produced the respective deficient diets (provided in pellets by Ssniff (Soest, FRG). Tab. 1 provides the Mg, Ca, Fe and vitamin E contents of the diets. Food and deionized water were provided ad libitum. The rats (5 per cage Type III) were kept at a light-dark cycle from 8 a.m. to 8 p.m. at a temperature of 21 ± 1 °C and a relative humidity of 50–60 % for 11 weeks. Body weight was measured weekly.
At the end of the experiment the rats were anesthesized with nembutal (50 mg/kg i.p.) and blood, liver, kidneys, heart, testes and aorta were taken, frozen in liquid nitrogen and stored at – 20 °C.
Intestines were inspected for intestinal tumors [17]. Thymus was inspected for malignent T cell lymphoma [18], removed and weighed. Leucocyte content was counted in lymphoma bearing rats by means of a Neubauer chamber.
Blood was centrifuged at 1000 g for 5 min. The concentrations of Mg, Ca, and Fe in serum were measured by atomic absorption spectrophotometry (AAS).
Portions of the livers and hearts were freeze-dried. Dried liver was powdered in a plastic mortar, freeze-dried hearts were powdered by means of a vibrating steel ball (Mikro-Dismembrator, B. Braun, Melsungen, FRG). For determination of Na, K, Mg, Ca, and Fe in livers and hearts, freeze-dried, powdered tissue was ashed in the Plasma Processor 200-E (Technics, München, FRG). The ash was dissolved in 0.1 N HCl. Na and K were measured by flame photometry (Klina, Beckman), Mg, Ca and Fe were measured by AAS (Philips, SP 9).
An aliquot of freeze-dried, powdered hearts was taken for determination of collagen content by means of measurement of hydroxyproline according to *Stegemann* [19].

Tab. 1: Content of Mg, Ca, Fe and vitamin E in control (A), Mg-deficient (B), Mg-deficient plus vit E-reduced (C) and Mg-deficient-vit E-supplemented (D) diets.

	A	B	C	D
Mg (mmol/kg)	26.5	4.3	4.7	3.1
Ca (mmol/kg)	272	249	249	247
Fe (mmol/kg)	3.24	3.29	2.97	3.10
α-Tocopherol (µmol/kg)	325	325	92	6965

Vitamin E was determined by its fluorescence in hexane extracts according to *Taylor* et al [20].
Malondialdehyde (MDA) was determined by a variation of the thiobarbituric acid (TBA) method [21, 22]. A 20 % homogenate in 150 mmol/l KCl (or serum) was diluted 1:1 (v/v) with 5 % trichloroacetic acid (TCA) and centrifuged for 5 min at 13 000 x g. 500 µl TBA (1 %, pH 7) was added to 500 µl supernatant and heated at 95 °C for 15 min. After cooling, the probes were extracted with 3 ml 1-butanol by vortexing for 30 sec and centrifugation at 2100 g for 15 min. MDA in the butanol phase was measured fluorometrically (Perkin Elmer LS 50, excitation: 532 nm, emission: 553 nm, slit width: 5 nm).
The calibration curve was prepared with malonaldehyde tetraethylacetal (Sigma), which was treated in the same way. Statistical analysis was performed as indicated in the tables.

Results and Discussion

Vitamin E content (tab. 2)

In serum and all investigated tissues, vitamin E content was reduced by Mg deficiency. This reduction was especially pronounced in serum and liver. When Mg deficiency was combined with vitamin E reduction, the vitamin E content was drastically reduced, particularly in liver. After feeding the vitamin E-supplemented diet to Mg-deficient rats, vitamin E content in serum and tissues was somewhat higher than in controls. However, in liver, vitamin E was markedly increased.
These results indicate that liver cells store vitamin E, and this store can be more depleted in vitamin E deficiency as compared with other tissues.
Dietary Zn deficiency also reduces the vitamin E content of rat serum, the only source studied [23]. The authors suggested that absorption and transport of tocopherol by the intestinal mucosa and the blood transport system may be affected [23]. However,

Tab. 2: Vitamin E content of serum (in µmol/l α-tocopherol) and various tissues (in µmol α-tocopherol/kg wet weight) of control (A), Mg-deficient (B), Mg-deficient plus vitE-reduced (C) and Mg-deficient-vitE-supplemented (D) rats.

	A	B	C	D
Serum	42.6 ± 3.7 (10)	26.0 ± 2.4 (10)^b	5.92 ± 0.67 (10)^c	50.8 ± 3.7 (10)
Liver	111.7 ± 7.2 (6)	63.0 ± 1.8 (6)^c	4.30 ± 1.30 (6)^c	465.5 ± 53.4 (6)^c
Heart	108.5 ± 6.5 (6)	83.2 ± 3.6 (6)^b	19.92 ± 2.30 (6)^c	135.0 ± 7.3 (6)^a
Kidney	57.0 ± 2.9 (6)	46.0 ± 1.6 (6)^b	10.74 ± 1.76 (6)^c	75.7 ± 4.9 (6)^b
Testis	77.1 ± 4.8 (8)	58.0 ± 3.8 (10)^b	16.69 ± 1.97 (6)^c	82.2 ± 6.2 (6)
Skeletal muscle	43.1 ± 2.2 (10)	32.1 ± 1.8 (10)^b	10.24 ± 1.28 (6)^c	65.5 ± 4.7 (6)^b

Mean ± SEM. Number of rats in brackets. Significant differences to control rats by unpaired Student's t-test.
a, $p < 0.05$; b, $p < 0.01$; c, $p < 0.001$.

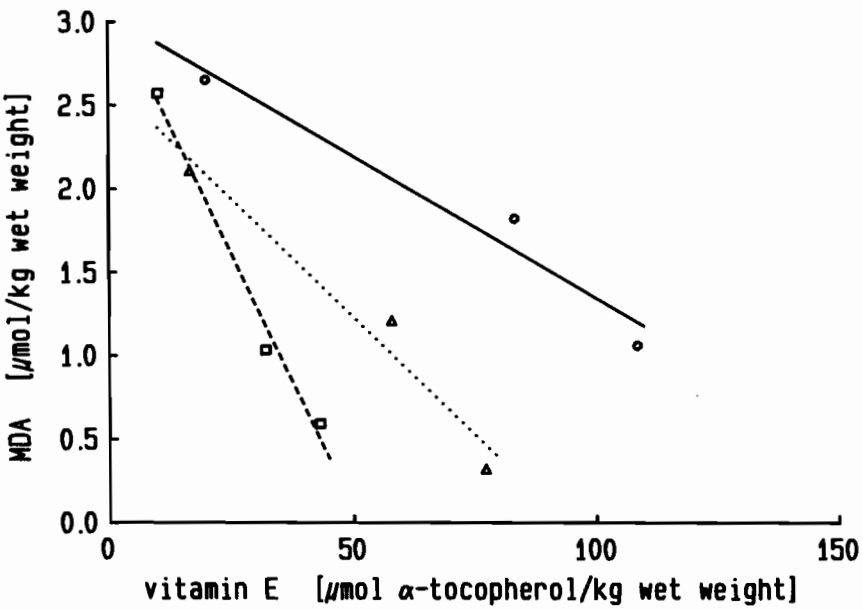

Fig. 1: Negative correlation between MDA (LPO) and vitamin E content of heart (○, r = –0.9765), testis (Δ, r = – 0.9789) and skeletal muscle (□, r = -0.9926). Data represent means of group A, B and C from Table 2 and 3.

Tab. 3: Malondialdehyde content of serum (in µmol/l) and various tissues (in µmol/kg weight) of control (A), Mg-deficient (B), Mg-deficient plus vitE-reduced (C) and Mg-deficient-vitE-supplemented (D) rats.

	A	B	C	D
Serum	0.071 ± 0.012 (8)	0.127 ± 0.008 (10)^b	0.108± 0.006 (10)^a	0.088 ± 0.005 (10)
Liver	0.70 ± 0.05 (6)	2.03 ± 0.42 (6)^a	15.34 ± 2.63 (6)^c	0.80 ± 0.08 (6)
Heart	1.06 ± 0.03 (6)	1.82 ± 0.22 (9)^b	2.65 ± 0.35 (10)^c	1.35 ± 0.14 (8)
Kidney	8.25 ± 0.82 (10)	23.16 ± 2.50 (10)^c	34.68 ± 3.58 (10)^c	10.99 ± 1.69 (10)
Testis	0.32 ± 0.02 (8)	1.21 ± 0.20 (8)^c	2.11 ± 0.31 (6)^c	0.31 ± 0.06 (6)
Skeletal muscle	0.59 ± 0.04 (9)	1.03 ± 0.17 (10)^b	2.57 ± 0.60 (6)^b	0.99 ± 0.12 (8)^b

Mean ± SEM. Number of rats in brackets. Significant differences to control rats by unpaired Student's t-test.
a, $p < 0.05$; b, $p < 0.01$; c, $p < 0.001$.

our results on MDA (tab. 3), showing the reciprocal behavior of vitamin E and MDA (fig. 1), suggests rather that reduction of vitamin E is caused by its destruction due to free radicals. Since lipid peroxidation due to oxygen free radicals is also increased by Zn deficiency [24, 25], the reduction of plasma vitamin E by Zn deficiency may also be caused by its destruction due to free radicals. Supporting this concept is the increased formation of free radicals by Fe-loading, and the increased cardiac and hepatic Fe content in Mg deficiency (see tab. 8, 12).

Malondialdehyde content (tab. 3)

MDA content, which was much higher in kidney than in other tissues, was increased by Mg deficiency (group B), particularly when also low in vitamin E (group C). After vitamin E supplementation of Mg-deficient rats (group D), MDA content was reduced to control values. Thus, MDA is reciprocally correlated to the free radical scavenger vitamin E, indicating increased formation of oxygen free radicals and increased LPO in Mg deficiency, which can be normalized by excess vitamin E. Alternatively, the formation of oxygen free radicals may be unchanged but scavenging of oxygen free radicals may be reduced in Mg deficiency. So far, there is no indication for this possibility. In any case, there is an increased action of oxygen free radicals in Mg deficiency. A more detailed analysis of the MDA and vitamin E contents of the tissues from group A, B and C revealed an excellent negative correlation between MDA and vitamin E for vitamin E contents up to 50–110 µmol/kg wet weight, depending on the tissue (fig. 1). The negative correlation was much less profound when the values of vitamin E-supplemented rats were included (data not shown). It appears, thus, that with high tissue vitamin E levels (exceeding a threshold), there is not a further effect on MDA production. Increased oxygen radical formation and LPO in Mg deficiency may be mediated by increased intracellular contents of Fe and Ca, increased release of catecholamines and

changed contents of polyunsaturated fatty acids of membrane phospholipids. Moreover, in Mg deficiency the synthesis of prostaglandins, particularly of thromboxane A_2, in association with increased formation of oxygen radicals, was increased. For detailed literature and discussion of these pathobiochemical mechanisms see Ref. [6, 7, 8].

The MDA levels in serum and tissues represent steady state concentrations and not formation of MDA, because MDA is rapidly metabolized by mitochondrial aldehyde dehydrogenase and is excreted by the kidneys [27,28]. Moreover, the formation of free radicals in Mg deficiency may be higher than indicated by LPO and MDA formation, because a part of free radicals was scavenged by vitamin E, leading to destruction of vitamin E in Mg deficiency (tab. 2 see above). Oxygen free radicals or lipid peroxyl radicals transform vitamin E to its phenoxy radical [29], followed by resonance stabilization [30] or secondary reactions of the vitamin E radical. Probably, renal excretion of MDA and enrichment of MDA (potentially by binding to protein) may be responsible for the high level of MDA in kidneys.

Comparison of our MDA values with those of other investigators [31,32,33] showed, that in serum they were lower by the factor 80 [31] or 50 [32, 33] and in liver by the factor 5 [33]. In an extensive discussion on MDA determination it was stated that MDA concentration in plasma is less than 0.1 µmol/l [34] as found by our method.

Of particular importance is the question whether LPO or some of its products may contribute to the pathological effects of Mg deficiency.

As shown with cultured fibroblasts, the products of LPO such as linoleic acid peroxide, MDA and 4-hydroxynonenal are cytotoxic at concentrations above 10^{-7} mol/l and vitamin E can protect against peroxidation damages [35].

In hepatocytes, LPO can change the functions of verapamil- and nifedipine-sensitive Ca^{2+} channels, resulting in a net influx of Ca^{2+}, an increase in $[Ca^{2+}]_i$ and sequestration of Ca^{2+} in the intracellular compartments [36]. This effect may be caused either by altered phospholipids (e.g. lysophosphatides) within the cell membrane or by the products of LPO.

Increased generation of oxygen radicals decreased the Ca content of cardiac sarcoplasmic reticulum by enhancing passive Ca permeability of this organelle [26, 37]. In heart muscle cells, oxygen free radicals increased $[Ca^{2+}]_i$ via a rise in Na^+/Ca^{2+} exchange and altered the electrical function of myocardial cells [38]. On the other hand, an increase in Na^+/Ca^{2+} can enhance the generation of oxygen free radicals [39]. At reduced extracellular Mg concentration as in Mg deficiency this increase in $[Ca^{2+}]_i$ can occur via an increase in $[Na^+]_i$ and/or by altered extracellular Mg^{2+}/Ca^{2+} interaction [40, 41, 42].

Thus, Mg deficiency may increase the action of oxygen free radicals and LPO by various mechanisms leading to increased $[Ca^{2+}]_i$ [14], and on the other hand, increased $[Ca^{2+}]_i$ can increase LPO [12].

The effects of LPO with respect to increased Ca^{2+} influx are similar to the effects of Mg deficiency.

Thus, some of the effects of Mg deficiency may be caused by increased LPO. When this mechanism plays a role in Mg deficiency, it can be expected that the effects of Mg deficiency are augmented by additional vitamin E reduction and may be reduced by additional vitamin E supplementation.

To test the premise that free radicals and LPO influence Mg-deficiency-induced changes, the effect of vitamin E deficiency and excess, in the presence of Mg deficiency was determined.

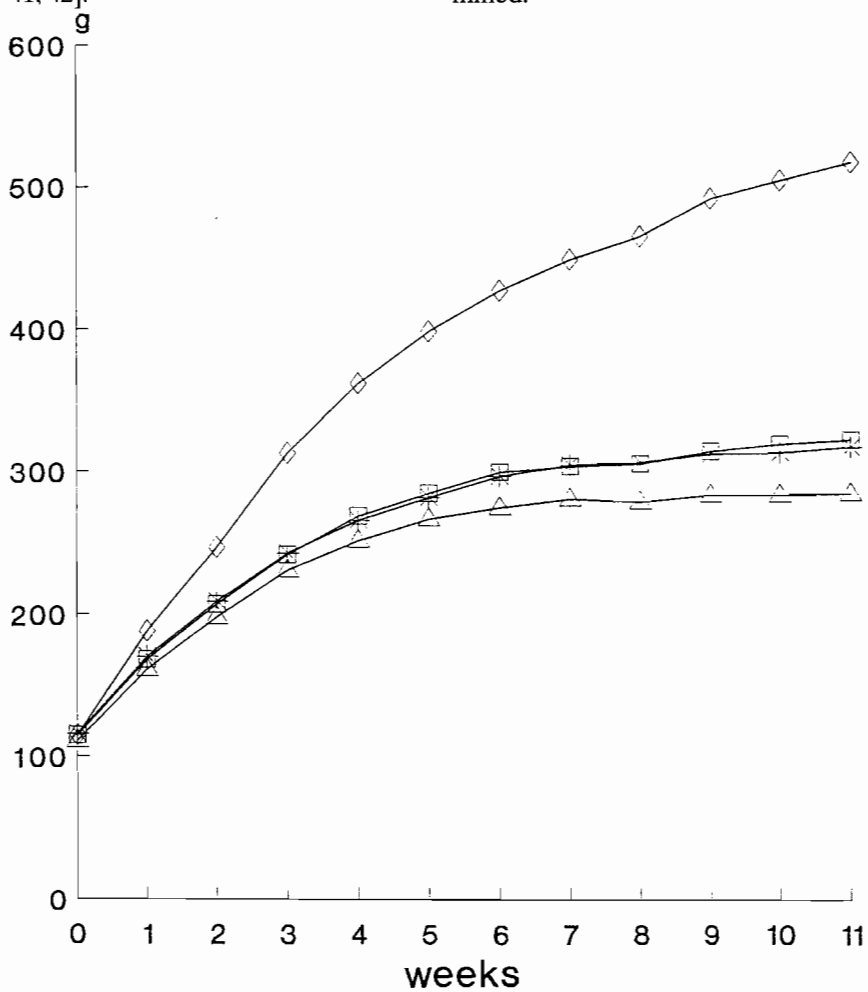

Fig. 2: Growth of rats fed control (◇), Mg-deficient (□), Mg-deficient plus vitamin E-reduced (✳) and Mg-deficient-vitamin E-supplemented (Δ) diet.

Erythema

When serum Mg concentration was drastically reduced after feeding an Mg-deficient diet to rats, the animals developed edema due to liberation of histamine from mast cells [43].
In the present experiments all rats developed erythema (seen particularly from red ears) with a peak at day 7 after starting with the Mg-deficient diet, and at day 12 the erythema had disappeared in all groups (data not shown).
There was no significant difference in erythema development among the experimental groups, indicating that erythema may be caused by altered extracellular Mg-Ca antagonism and Ca-dependent histamine release, independent of oxygen free radicals.

Body weight (fig. 2)

Since the study explored only whether the effects of Mg deficiency can be influenced by vitamin E, additional pair-fed groups were omitted. Weight gain was severely reduced in Mg deficiency. Additional reduction of vitamin E did not significantly change body weight, indicating that the Mg content of the diets was the growth-limiting factor.
In agreement with this conclusion, the growth-rate of the vitamin E-supplemented Mg-deficient group was somewhat lower, because the Mg content of the vitamin E-supplemented Mg-deficient diet was somewhat lower than in the other diets (tab. 1).

Tab. 4: Body weight, weight of thymus and left testis of control (A), Mg-deficient (B), Mg-deficient plus vitE-reduced (C) and Mg-deficient-vitE-supplemented (D) rats at the end of the experiment.

	A	B	C	D
Body weight	520 ± 2	306 ± 2 ^{c}	316 ± 2 ^{c}	281 ± 2 ^{c}
Thymus	0.85 ± 0.02	0.46 ± 0.01 ^{c}	0.47 ± 0.01 ^{c}	0.40 ± 0.01 ^{c}
Testis	1.73 ± 0.01	1.95 ± 0.02 ^{c}	2.01 ± 0.01 ^{c}	2.12 ± 0.02 ^{c}

Values in g. Mean ± SEM of 20 rats in each group. Significant differences to control rats by unpaired Student's t-test.
c, $p < 0.001$.

Weight of thymus and testis (tab. 4)

During Mg deficiency the weight of the thymus was reduced, but the body weight/thymus weight remained constant.
The weight of the testes of Mg-deficient and Mg-deficient plus vitamin

Tab. 5: Skin ulcerations of control (A), Mg-deficient (B), Mg-deficient plus vitE-reduced (C) and Mg-deficient-vitE-supplemented (D) rats.

	A			B			C			D		
day	0	1	2	0	1	2	0	1	2	0	1	2
0	0/25			0/50			0/50			0/50		
14	24/24	0/24	0/24	41/49	5/49	3/49^{b}	42/49	4/49	3/49^{c}	43/46	3/46	0/46
24	24/24	0/24	0/24	42/49	4/49	3/49^{a}	35/47	9/47	3/47^{e}	44/46	1/46	1/46^{g}
31	24/24	0/24	0/24	43/49	3/49	3/49^{a}	37/47	6/47	4/47^{d}	44/46	1/46	1/46^{f}
36	24/24	0/24	0/24	42/49	6/49	1/49^{a}	39/47	4/47	4/47^{d}	43/46	2/46	1/46^{f}
42	24/24	0/24	0/24	43/49	4/49	2/49^{a}	38/47	6/47	3/47^{d}	42/44	1/44	1/44^{f}

0, no ulcerations; 1, light ulcerations; 2, severe ulcerations. Values represent number of rats / total number of rats. Differences to a total of 25 or 50 reflects number of dead rats per group.

For statistical analysis light and severe ulcerations were taken together. Statistical analysis was performed according to Chi²-test.
Significance between group A and B: a, $p < 0.10$ b, $p < 0.05$
Significance between group A and C: c, $p < 0.10$ d, $p < 0.05$ e, $p < 0.01$
Significance between group C and D: f, $p < 0.05$ g, $p < 0.01$

Differences between group B and C were not significant

Tab. 6: Development of thymus lymphoma and abdominal tumors.
A, Control. B, Mg-deficient rats. C, Mg-deficient plus vitE-reduced rats. D, Mg-deficient-vitE-supplemented rats.

Group	Weight of lymphoma (g)	Weight of spleen (g)	Number of leucocytes (μl^{-1})	Diameter of abdominal tumor (cm)
A	-	-	-	-
B	-	-	-	-
C	10.3	3.1	5.800	-
	10.0	4.7	14.000	-
	-	-	3.500	1.5
	-	-	3.200	1.0
D	4.7	0.8	4.200	-
	9.5	4.4	80.000	-
	8.3	3.5	21.600	-
	-	-	3.300	1.5

In group A and B, there were no tumors. The values in the same line belong to the same rat. (2 rats with lymphoma and 2 rats with abdominal tumors in group C, 3 rats with lymphoma and 1 rat with abdominal tumor in group D).

E-reduced rats was not reduced but slightly increased. Since reduction of testis weight is characteristic of vitamin E deficiency [44], this result shows that the vitamin E content of the vitamin E-reduced diet was still sufficient to prevent classic vitamin E deficiency symptoms, although the reduced contents of vitamin E in serum and tissues of group C led to a further increase in MDA compared to group B.

Skin lesions (tab. 5)

During Mg deficiency, some of the Mg-deficient rats (group B) developed ulcerations of the skin, the pathologic mechanism of which is unknown. In the Mg-deficient plus vitamin E-reduced group (C) the number of ulcerations was higher than in group B and in the Mg-deficient vitamin E-supplemented group (D), the number of ulcerations was lower than in group B and C.
It appears that free radicals are involved in the development of skin ulcerations. Possibly, increased numbers of leucocytes, which produce free radicals [45], increase the need for vitamin E, which scavenge free radicals.

Development of thymus lymphoma (tab. 6)

In chronic severe experimental Mg deficiency, 13 % of the rats developed malignant T cell lymphoma of the thymus (18). In the present experiment the rate of lymphoma was lower (3.5 %), the reasons probably being the following:
In our preceding experiments we used female rats, whose body weight at start of the experiment was lower (70–80 g) and a more severe Mg deficiency had been induced.
In parallel to the development of thymus lymphoma, the spleen was enlarged and the number of leucocytes in blood was increased. Since the vitamin E-supplemented Mg-deficient rats (group D) had even more thymus lymphomata than did the Mg-deficient vitamin E low rats (group C), it appears that free radicals do not play a role in the development of Mg deficiency-induced thymus lymphoma.

Development of tumors (tab. 6)

In preceding experiments [17] a tumor-like connective tissue proliferation was observed in the intestine of Mg-deficient rats. In the present experiment with less severe Mg deficiency, we did not find this alteration.
However, we observed 3 tumors originating from the muscle layer of the abdominal wall. There was also one tumor in Mg-deficient vitamin E-supplemented rats. Therefore, it remains open whether the development of these tumors is dependent on free radicals and MDA, which have been implicated in tumor development [27, 46]. The mechanism of tumor development in Mg deficiency is unknown.

Electrolyte and mineral content of serum and tissues

Serum (tab. 7)

Mg concentration of all groups, fed the Mg-deficient diet was markedly reduced, indicating severe Mg deficiency. Serum Ca concentration was not significantly changed, but serum Fe was somewhat reduced (tab. 7). Since the Fe content of some tissues was increased (tab. 8, 9, 12) and Fe excretion is minimal, the reduction of serum Fe by Mg deficiency may be caused by intracellular Fe uptake. Possibly, cellular Fe uptake which is performed by internalization of the Fe transferrin complex [47] is increased in Mg deficiency.

Tab. 7: Mg, Ca and Fe concentration in serum of control (A), Mg-deficient (B), Mg-deficient plus vitE-reduced (C) and Mg-deficient-vitE-supplemented (D) rats.

	A	B	C	D
Mg (mmol/l)	0.75 ± 0.02 (10)	0.21 ± 0.01 (29)^{c}	0.18 ± 0.01 (25)^{c}	0.17 ± 0.01 (23)^{c}
Ca (mmol/l)	2.47 ± 0.05 (10)	2.53 ± 0.02 (10)	2.47 ± 0.01 (10)	2.46 ± 0.08 (10)
Fe (µmol/l)	39.9 ± 1.4 (23)	33.3 ± 2.3 (11)^{a}	31.0 ± 3.0 (12)^{a}	32.9 ± 3.0 (12)^{a}

Mean ± SEM. Number of rats in brackets. Significant differences to control rats by unpaired Student's t-test.
a, $p < 0.05$; c, $p < 0.001$.

Tab. 8.: K, Mg, Ca, Fe and hydroxyproline content in heart of control (A), Mg-deficient (B), Mg-deficient plus vitE-reduced (C) and Mg-deficient-vitE-supplemented (D) rats.

	A	B	C	D
K	195 ± 3 (6)	170 ± 3 (10)^{c}	182 ± 3 (9)^{b}	175 ± 4 (10)^{b}
Mg	36.91 ± 0.35 (8)	34.42 ± 0.39 (10)^{c}	34.31± 0.37 (9)^{c}	33.69 ± 0.37 (10)^{c}
Fe	6.12 ± 0.15 (8)	6.84 ± 0.09 (10)^{c}	6.94 ± 0.10 (9)^{c}	7.09 ± 0.16 (10)^{c}
Ca	1.93 ± 0.07 (8)	2.07 ± 0.04 (10)	2.11 ± 0.06 (9)	2.14 ± 0.03 (10)^{a}
Hydroxy-proline	1.50 ± 0.11 (8)	1.49 ± 0.15 (8)	1.40 ± 0.13 (6)	1.49 ± 0.17 (9)

Values in mmol/kg dry weight, for hydroxyproline in mg/g dry weight. Mean ± SEM. Number of rats in brackets. Significant differences to control rats by unpaired Student's t-test.
a, $p < 0.05$; b, $p < 0.01$; c, $p < 0.001$.

Heart (tab. 8)

During Mg deficiency, the contents of K and Mg were decreased by approximately 10 % and the contents of Fe and Ca were increased by approximately 10 %, confirming findings in other studies [48].
Neither low nor high dietary vitamin E influenced Mg-deficiency-induced alterations in mineral content. Therefore, under the conditions of the present experiment the increased level of oxygen free radicals did not lead to cardiac electrolyte alterations. A different result was found with Mg-deficient Syrian hamsters in which vitamin E supplementation could reduce the extent of cardiac lesions [9]. Probably, the Syrian hamster is more sensitive to Mg deficiency and LPO and/or may release more catecholamines than the rat strain used in the present experiment.
In agreement with a lack of severe cardiac injury in the present experiment, there was no alteration in cardiac hydroxyproline (collagen) content among the experimental groups (tab. 8). Preceding experiments only showed a strong increase in the myocardial hydroxyproline content, when Mg-deficient rats were additionally chronically stressed, thus increasing catecholamine release [49, 50, 51].

Aorta (tab. 9)

Cardiac injury may also result from ischemia resulting from vascular calcification and atherosclerosis such as has been induced by chronic Mg deficiency [52]. In addition, also LPO may play a role in atherosclerosis [10, 53].
As shown in tab. 9, Mg deficiency caused a 10 % decrease in cellular Mg content, a 20 % increase in Ca and a 10 to 30 % increase in Fe content.
However, vitamin E reduction and vitamin E supplementation had no significant influence on the Mg deficiency-induced alterations. From this result it can be concluded that under the conditions of the present experiment, LPO had no effect on the alterations of aortic mineral content.

Tab. 9: Mg, Ca and Fe content of aorta from control (A), Mg-deficient (B), Mg-deficient plus vitE-reduced (C) and Mg-deficient-vitE-supplemented (D) rats.

	A	B	C	D
Mg	7.00 ± 0.13 (24)	6.36 ± 0.14 (21)^{b}	6.34 ± 0.17 (20)^{b}	6.44 ± 0.12 (20)^{b}
Ca	8.86 ± 0.18 (24)	10.48 ± 0.31 (21)^{c}	10.77 ± 0.25 (21)^{c}	10.63 ± 0.27 (21)^{c}
Fe	2.40 ± 0.14 (22)	2.68 ± 0.23 (18)	3.10 ± 0.16 (19)^{a}	2.77 ± 0.22 (10)

Values in mmol/kg dry weight. Mean ± SEM. Number of rats in brackets. Significant differences to control rats by unpaired Student's t-test.
a, $p < 0.05$; b, $p < 0.01$; c, $p < 0.001$.

Kidney (tab. 10)

Renal calcification, a typical consequence of Mg deficiency in rats, was confirmed. Neither lowering dietary vitamin E nor providing an excess had a significant effect on kidney Ca content, indications that renal calcification is an effect of lowered Mg/Ca ratio, and is not caused by free radicals.

Skeletal muscle (tab. 11)

In Mg-deficient rats the skeletal muscle contents of Na and Ca were increased and the contents of K and Mg were decreased. These findings, which confirmed earlier studies (review: 48), were not influenced by vitamin E reduction or supplementation. Therefore, the alterations in mineral content which are caused by increased membrane permeability are not caused by free radicals.

Liver (tab. 12)

Mg deficiency-induced alterations of Na, K, Ca and Mg content in liver were less than in other tissues [8, 48] or did not occur at all [48]. However, the Mg deficiency-induced increase of Fe was greater in liver than in other tissues [8, 54].
In the present Mg deficiency experiment there were no significant alterations in Na, K, Mg and Ca content. However, the Fe content in liver was much higher than in preceding experiments, probably because of the longer experimental period (11 weeks compared to 2 weeks [54] or 4 weeks [8]).
Additional vitamin E reduction and vitamin E supplementation had no significant effect on Fe content. Thus, it can be concluded that it was the Mg deficiency, not the free radicals, that induced the increase in liver Fe. Possible mechanisms may be:

1. Increased instability and destruction of erythrocytes [55], the released Fe being stored in the liver.
2. Increased intestinal Fe absorption because in experiments with mouse duodenal fragments, addition of Mg inhibited ^{59}Fe uptake [56].

Tab. 10: Mg and Ca content of kidney from control (A), Mg-deficient (B), Mg-deficient plus vitE-reduced (C) and Mg-deficient-vitE-supplemented (D) rats.

	A	B	C	D
Mg	31.7 ± 0.7 (8)	30.3 ± 0.4 (10)	30.5 ± 0.5 (10)	30.6 ± 0.6 (10)
Ca	6.7 ± 0.4 (10)	33.6 ± 11.5 (6)[a]	56.0 ± 18.7 (9)[a]	118.7 ± 45.6 (7)[a]

Values in mmol/kg dry weight. Mean ± SEM. Number of rats in brackets. Significant differences to control rats by unpaired Student's t-test.
a, $p < 0.05$.

Tab. 11: Na, K, Mg and Ca content of skeletal muscle (M. gastrocnemius) from control (A), Mg-deficient (B), Mg-deficient plus vitE-reduced (C) and Mg-deficient-vitE-supplemented (D) rats.

	A	B	C	D
Na	90.8 ± 3.0 (9)	108.8 ± 4.9 (8)[b]	118.9 ± 7.5 (8)[a]	120.8 ± 3.3 (9)[c]
K	304.9 ± 9.9 (9)	268.4 ± 8.3 (9)[a]	257.1 ± 13.5 (9)[a]	266.3 ± 7.5 (7)[b]
Mg	40.7 ± 0.6 (10)	36.5 ± 1.4 (10)[a]	36.9 ± 0.9 (10)[b]	36 1 ± 1.0 (9)[b]
Ca	5.83 ± 0.57 (10)	6.76 ± 0.60 (10)	7.49 ± 0.38 (10)[a]	7.39 ± 0.38 (9)[a]

Values in mmol/kg dry weight. Mean ± SEM. Number of rats in brackets. Significant differences to control rats by unpaired Student's t-test.
a, $p < 0.05$; b, $p < 0.01$; c, $p < 0.001$.

3. Increased cellular non-transferrin-Fe (NT-Fe) uptake. Although NT-Fe in serum is less than 1 % of total Fe, it is more rapidly taken up by hepatocytes than transferrin-bound Fe [57]. NT-Fe uptake was effectively inhibited by Zn^{2+} and Mn^{2+} [57]. In analogy, at reduced extracellular Mg^{2+}, as in Mg deficiency, more NT-Fe may be taken up.
4. Increased uptake of Fe-transferrin by liver.

The slightly reduced serum Fe concentration in groups B, C, D is compatible with the last possibilities.
There was no correlation between MDA (tab. 3) and Fe in liver and heart (tab. 8, 12). Therefore, MDA content is not a simple function of Fe content and oxygen free radicals. However, the type of Fe binding (weakly bound Fe is essential for oxygen radical formation) and the availability of protective substances (e.g. vitamin E) determine the level of oxygen free radicals, LPO and MDA. The major part of Fe stored in the liver of Mg deficient rats may be bound as ferritin and hemosiderin which is less active in radical formation [58].
An additional finding was the different hepatic mineral content from Mg-deficient rats which had developed a thymus lymphoma (group E). In these livers, K, Mg and Fe content were increased and Ca content was reduced compared to Mg-deficient rats without thymus lymphoma. Further experiments are needed to explain this effect.

Conclusion

In Mg deficiency, the basic rate of MDA produced by LPO via oxygen free radicals was increased, depending on vitamin E nutrition. Mg deficiency reduced the content of vitamin E in serum and tissues, probably by an increased formation of free radicals. However, only in Mg deficiency-induced skin ulcerations, vitamin E and free radicals may be involved. There was a negative correlation between MDA, which is formed by oxygen free radicals, and vitamin E content in the tissues. Thus, in Mg deficiency and particularly in Mg deficiency plus vitamin E reduction, there

Tab. 12: Na, K, Mg, Ca and Fe content of liver from control (A), Mg-deficient (B), Mg-deficient plus vitE-reduced (C) and Mg-deficient-vitE-supplemented (D) rats. Group E contains livers from rats which had developed thymus lymphoma.

	A	B	C	D	E
Na	102.8 ± 8.1 (10)	112.4 ± 5.3 (10)	114.2 ± 8.0 (10)	116.8 ± 5 (10)	-
K	333.6 ± 3.9 (16)	343.7 ± 3.9 (12)	339.9 ± 4.7 (12)	342 ± 4.2 (11)	444.3 ± 3.6 (4)[c]
Mg	29.10 ± 0.25 (16)	28.81 ± 0.49 (12)	27.64 ± 0.47 (12)[b]	28.26 ± 0.31 (11)[a]	33.33 ± 1.56 (4)[c]
Ca	1.95 ± 0.09 (16)	1.88 ± 0.08 (12)	1.82 ± 0.06 (12)	1.85 ± 0.04 (11)	1.34 ± 0.06 (4)[b]
Fe	7.24 ± 0.39 (16)	21.13 ± 2.85 (12)[c]	16.37 ± 1.45 (12)[c]	19.26 ± 1.37 (11)[c]	34.89 ± 4.12 (4)[c]

Values in mmol/kg dry weight. Mean ± SEM. Number of rats in brackets. Significant differences to control rats by unpaired Student's t-test.
a, $p < 0.05$; b, $p < 0.01$; c, $p < 0.001$.

was an increased formation of MDA due to an increased action of oxygen free radicals. However, most effects of Mg deficiency were independent of vitamin E, although the level of vitamin E was in the range of correlation to MDA. This means, that there was an increased action of oxygen free radicals and formation of MDA in Mg deficiency, which had no pathological consequences within the experimental period. Probably, cells are able to repair LPO to some extent. Nevertheless, pathological effects which may be caused by oxygen free radicals, not investigated in the present experiment (e.g. aging [59]), may be enhanced by Mg deficiency.

Since Mg deficiency reduced vitamin E which is a major protective factor against free oxygen radicals and since some toxic mechanisms (e.g. CCl_4, ionizing radiation, stress, reperfusion injury etc.) are operating via increased production of free radicals and LPO, Mg deficiency is a risk factor for pathological effects working via free radicals and LPO.

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