Effects of Isoproterenol and Magnesium Deficiency on Vitamin E Content, Lipid Peroxidation and Mineral Metabolism of Various Tissues

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Zusammenfassung

Männliche Ratten wurden 24 Tage lang mit normaler und Mg-Mangeldiät gefüttert, welche zusätzlich einen normalen, verminderten oder erhöhten Vitamin E-Gehalt hatten.
Anschließend wurde der Hälfte der Ratten 3 mg/kg Isoproterenol s. c. injiziert und 4 Stunden später Blut, Leber, Nieren und Herz zur Bestimmung von Malondialdehyd (MDA), Vitamin E, Fe, Mg und Ca entnommen.
In den Geweben war MDA bis zu einem Vitamin E-Gehalt von 110 nmol/g Feuchtgewicht negativ mit Vitamin E korreliert. Oberhalb dieser Schwelle wurde MDA nicht mehr durch Vitamin E vermindert.
Mg-Mangel verringerte den Vitamin E-Gehalt in Plasma und Geweben und erhöhte den Fe-Gehalt besonders in der Leber.
Isoproterenol reduzierte das Plasma-Fe und erhöhte Mg in Plasma und Leber. Im Herzen reduzierte Isoproterenol den Mg-Gehalt und erhöhte den Ca-Gehalt. Die durch Isoproterenol verursachte Ca-Zunahme im Herzen wurde durch Vitamin E-Supplementation fast vollständig verhindert.

Summary

Male rats were fed normal and Mg-deficient diets for 24 days. These diets contained either normal, reduced or enhanced amounts of vitamin E. Thereafter, half of the rats were s. c. injected with 3 mg/kg isoproterenol and 4 hours later blood, liver, kidney and heart were taken for measuring the contents of malondialdehyde (MDA), vitamin E, Fe, Mg and Ca.
In the tissues, MDA was negatively correlated to vitamin E up to a vitamin E content of 110 nmol/g wet weight. Above this threshold, MDA was not further reduced by vitamin E.
Mg deficiency reduced vitamin E content in plasma and tissues and increased Fe content in tissues, particularly in liver.
Isoproterenol reduced plasma Fe and increased plasma and liver Mg. In the heart isoproterenol reduced Mg and increased Ca. The increase in cardiac Ca by isoproterenol was almost completely prevented by vitamin E supplementation.

Résumé

Des rats mâles ont reçu, pendant 24 jours, des régimes alimentaires à teneur normale ou réduite en Mg. En outre, ces régimes contenaient des proportions normales, réduites ou accrues de vitamine E. La moitié des animaux a ensuite été traitée par une injection sous-cutanée de 3mg/kg d'isoprénaline puis les rats ont été sacrifiés 4 heures plus tard et on a enregistré les concentrations de malone-dialdéhyde (MDA), de vitamine E, de Fe, de Mg et de Ca dans le sang, le foie, le rein et le coeur.
Les taux tissulaires de MDA et de vitamine E ont été inversement corrélés, jusqu'à une teneur de 110 nmol de vitamine E par gramme de poids frais. Au-delà de ce seuil, l'adjonction de vitamine E n'a pas accru la diminution du taux de MDA.
La carence en Mg a diminué les concentrations plasmatiques et tissulaires de vitamine E et a augmenté les taux tissulaires de Fe, en particulier au niveau hépatique.
L'isoprénaline a réduit le taux plasmatique de Fe et accru les concentrations plasmatiques et hépatiques de magnésium. Au niveau cardiaque, l'isoprénaline a diminué le taux de Mg et augmenté celui de Ca. Cette augmentation du Ca cardiaque par l'isoprénaline a été quasiment abolie par la supplémentation en vitamine E.

Introduction

Isoproterenol induces infarct-like myocardial damage [1]. Several hypotheses have been postulated to explain the mechanism of injury. These include hypoxia [1], increased influx of Ca [2, 3] followed by depletion of ATP [3] and a rise in cAMP [4]. Another toxic mechanism of isoproterenol may be oxygen free radicals, which are formed during the oxidation of isoproterenol and other catecholamines to orthoquinones and adrenochromes [5, 6, 7].
Oxygen free radicals can react with nucleic acids, proteins, sugars and particularly with polyunsaturated fatty acids of phospholipid membranes, resulting in lipid peroxidation (LPO) [8]. The latter mechanism and its products may lead to increased membrane permeability and, when exceeding a critical level, to cell injury [9, 10, 11].
Usually, oxygen free radicals are formed by Fe-dependent reduction of O_2. The toxic effects of oxygen radicals can be reduced by various protective mechanisms, e.g. superoxide dismutase, catalase, glutathione peroxidase, vitamin E, vitamin C [8, 12]. On the other hand, the cellular Fe content, particularly in liver cells, and LPO can be enhanced by Mg deficiency [13, 14], and Mg deficiency-induced myocardial injury in hamsters has been reduced by vitamin E [15].
Therefore, we investigated the effects of isoproterenol, Mg deficiency and vitamin E on LPO, as an indicator of oxy-

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Tab. 1: Vitamin E and Mg content of the diets.

	Group	Vit. E mmol/kg	Mg mmol/kg
Control, -	I	0.42	22.6
" , Vit. E red.	II	0.20	22.6
" , Vit. E suppl.	III	32.70	22.6
Mg def., -	IV	0.40	1.9
" , Vit. E red.	V	0.23	1.9
" , Vit. E suppl.	VI	31.20	1.9

gen radical formation, and on mineral metabolism, as an indicator of tissue injury.

Material and Methods

Having been approved by local authorities and the Animal Protection Committee the experiment was performed with male Wistar rats (Interfauna, Tuttlingen, FRG) weighing 95–110 g. Male rats were taken, because it has been reported that male rats may be more susceptible to hepatotoxicity by oxygen free radicals [16]. The rats were fed with a control or Mg-deficient diet, which were additionally reduced by vitamin E or supplemented with vitamin E according to tab. 1. The diets (pellets) were obtained from Ssniff (Soest, FRG). The composition of the control diet has been described in detail [17]. Mg-deficient and vitamin E-reduced diets were produced by omission of Mg and vitamin E from the mineral or vitamin mix. The rats were fed the diets and demineralized water ad libitum for 24 days.

Thereafter, half of the rats in the 6 dietary groups were s.c. injected with 3 mg/kg (–)-isoproterenol hydrochloride (Sigma). Four hours after injection the rats were anesthetized by i.p. administration of 60 mg/kg pentobarbital.

Blood, liver, kidneys and heart were taken, and the tissues were frozen in liquid nitrogen and stored at –20 °C.

Heparinized blood was centrifuged at 1000 g for 5 min. The concentrations of Mg, Ca and Fe in plasma were measured by atomic absorption spectrophotometry (AAS, Philips, SP9).

A part of the livers and one kidney were freeze-dried. Powdered tissue was ashed in the Plasma Processor 200-E (Technics, München, FRG). The ash was dissolved in 0.1 N HCl and Fe, Mg and Ca were measured by AAS.

Vitamin E in plasma and tissues was determined by its fluorescence in hexane extracts according to *Taylor* et al. [18]. For calibration D, L α-tocopherol (Serva) was used.

Malondialdehyde (MDA) was determined by a variation of the thiobarbituric acid (TBA) method [19, 20]. A 20% homogenate of the tissues in 150mmol/l KCl was diluted 1 : 1 (v/v) with 5% trichloroacetic acid and centrifuged for 5 min at 13000 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 malondialdehyde tetraethylacetal (Sigma), which was treated in the same way. The hearts were homogenized. An aliquot of the 20% homogenate was taken for measurement of vitamin E and MDA.

The remaining homogenates were freeze-dried, ashed in the Plasma Processor and taken for measurement of Ca, Mg and Fe by AAS, as described above.

Results and Discussion

Physical Development and Mortality

Rats kept on diets with normal Mg content (group I–III) developed normally, i.e. vitamin E supply did not affect growth. All groups receiving the Mg-deficient diets (groups IV–VI) developed typical erythema during days 6 to 10. On day 24, body weight was one third lower in all Mg-deficient groups (IV–VI). Following s.c. injection of isoproterenol, 75% of the rats fed the normal Mg content in the diets (groups I–III) died, whereas in the Mg-deficient groups (IV–VI) mortality was only 25%. Death usually occurred within 10 to 20 min after injection and was associated with lung edema. This result can be explained by the Mg-dependency of isoproterenol binding to β-adrenergic receptors, which was enhanced 5-fold by Mg^{2+} [21, 22]. Halfmaximal effect of Mg^{2+} was obtained at 0.4 mmol/l Mg^{2+} with receptors from frog erythrocytes [21] and at 2–3 mmol/l Mg^{2+} with receptors from lymphoma cells [22]. Thus, the drastic reduction of extracellular Mg^{2+} by Mg deficiency had reduced receptor affinity to isoproterenol and death rate.

The death rate in the groups with different vitamin E content of the food (data not shown) was not significantly different, indicating that free oxygen radicals are not involved in acute toxicity of isoproterenol.

Vitamin E and Malondialdehyde (tab. 2, 3)

As discussed elsewhere [13], malondialdehyde (MDA) values in plasma measured by the TBA method can be doubted and were therefore not included.

After feeding the vitamin E-reduced (II) or Mg-deficient (IV) diets, MDA content was increased in liver, heart and kidney. When both deficiencies concurred, MDA was further increased and behaved almost additively in all tissues (V). Thus, MDA and vitamin E are negatively correlated (fig. 1).

Vitamin E excess in the normomagnesemic diet had no effect on MDA (group III) compared to the controls. Also vitamin E excess in the Mg-deficient diet (VI) did not prevent the increase of MDA due to Mg deficiency.

Injection of isoproterenol increased MDA in liver and heart in groups I, II, IV and V but not in groups III and VI. These results indicate that isoproterenol can enhance the formation of oxygen free radicals and LPO and excess of vitamin E may scavange oxygen radicals induced by isoproterenol.

Feeding a vitamin E-reduced (group II) or Mg-deficient diet (group IV) decreased vitamin E content of plasma. When both deficiencies concurred (V), vitamin E was not further reduced.

Tab. 2: Malondialdehyde content in various tissues (in µmol/kg wet weight).

Group	n	Liver	Kidney	Heart
I, -	4	0.84 ± 0.13	3.30 ± 0.22	1.03 ± 0.17
I, Iso	4	1.48 ± 0.11^{e}	3.30 ± 0.46	1.75 ± 0.19^{d}
II, -	4	1.48 ± 0.10^{b}	8.37 ± 1.92^{a}	2.65 ± 0.15^{c}
II, Iso	6	2.66 ± 0.43^{d}	7.68 ± 1.33	3.61 ± 0.22^{e}
III, -	5	0.73 ± 0.06	3.02 ± 0.13	1.15 ± 0.14
III, Iso	4	0.71 ± 0.01	3.22 ± 0.12	1.47 ± 0.23
IV, -	5	1.48 ± 0.14^{b}	4.22 ± 0.15^{b}	1.80 ± 0.22^{a}
IV, Iso	8	2.40 ± 0.37^{d}	4.86 ± 0.47	2.92 ± 0.21^{e}
V, -	6	2.92 ± 0.30^{c}	9.75 ± 1.17^{c}	3.23 ± 0.20^{c}
V, Iso	7	5.29 ± 0.74^{d}	9.89 ± 1.86	4.21 ± 0.26^{d}
VI, -	5	1.44 ± 0.19^{b}	5.68 ± 0.75^{a}	1.77 ± 0.14^{a}
VI, Iso	5	1.67 ± 0.32	5.74 ± 0.72	1.92 ± 0.14

Control (I), vitamin E-reduced (II), vitamin E-supplemented (III), Mg-deficient (IV), Mg-deficient + vitamin E reduced (V), and Mg-deficient-vitamin E-supplemented (VI) rats. A part of the rats in each group were s.c. injected with 3 mg/kg isoproterenol (ISO), n = number of rats, Mean ± SEM. Significant differences were calculated by unpaired Student's t-test.
Significant differences to controls. [a], $p < 0.05$; [b], $p < 0.01$; [c], $p < 0.001$. Significant differences between isoproterenol-treated and untreated rats within each group; [d], $p < 0.05$; [e], $p < 0.01$; [f], $p < 0.001$.

Tab. 3: Vitamin E content of plasma (in µmol/l α-tocopherol) and various tissues (in µmol α-tocopherol/kg wet weight). See legend to tab. 2.

Group	n	Plasma	Liver	Kidney	Heart
I, -	4	43.1 ± 0.7	91.0 ± 5.3	66.2 ± 3.1	110.1 ± 3.9
I, Iso	4	31.7 ± 0.5^{f}	87.7 ± 0.3	65.5 ± 3.8	110.5 ± 6.6
II, -	4	11.6 ± 0.8^{c}	18.9 ± 0.5^{c}	20.6 ± 1.2^{c}	34.5 ± 2.2^{c}
II, Iso	6	9.2 ± 0.4^{d}	20.1 ± 0.9	22.4 ± 1.5	31.0 ± 1.2
III, -	5	83.7 ± 1.2^{c}	824 ± 100^{c}	92.3 ± 2.1^{c}	193.0 ± 11.5^{c}
III, Iso	4	52.5 ± 4.3^{f}	687 ± 76	88.7 ± 3.5	188.9 ± 14.3
IV, -	5	29.3 ± 1.2^{c}	59.1 ± 5.2^{c}	55.4 ± 2.8^{a}	91.7 ± 5.5^{a}
IV, Iso	8	23.8 ± 0.3^{e}	55.6 ± 3.2	55.0 ± 2.0	84.6 ± 3.1
V, -	6	12.0 ± 0.4^{c}	19.3 ± 1.1^{c}	27.8 ± 1.0^{c}	42.5 ± 1.5^{c}
V, Iso	7	11.5 ± 0.4	20.8 ± 1.3	26.9 ± 0.9	39.9 ± 1.7
VI, -	5	62.3 ± 3.0^{c}	356 ± 47^{c}	98.8 ± 5.0^{c}	174.0 ± 13.5^{b}
VI, Iso	5	47.8 ± 2.5^{e}	327 ± 44	94.6 ± 2.5	174.1 ± 11.6

After feeding the vitamin E-supplemented diets (III, VI), vitamin E contents were increased in plasma.
Vitamin E contents in liver, kidney and heart behaved in analogy to vitamin E content in plasma. Mg deficiency also reduced vitamin E contents in all tissues. This effect of Mg deficiency was particularly expressed in plasma and liver.
Injection of isoproterenol reduced vitamin E in plasma and had no significant effect on vitamin E content in the tissues.

A most remarkable result was the depletion of vitamin E in plasma and tissues by Mg deficiency. Similarly, also in dietary Zn deficiency vitamin E content of rat plasma was reduced [23]. Other tissues were not investigated by these authors [23]. As an explanation it was discussed that absorption and transport of tocopherol by the intestinal mucosa and the blood transport system may be affected [23].
However, from our results on MDA (fig. 1), showing the reciprocal behaviour of vitamin E and MDA, it appears to be more likely that reduction of vitamin E is caused by its destruction due to free radicals. Since LPO 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. In agreement with this conclusion, iron loading, too, which leads to increased formation of oxygen free radicals caused a reduction of vitamin E [26]. In agreement with this mechanism the Fe content in liver, heart and kidney was increased by Mg deficiency (tabs. 5, 6, 7).
Besides Mg deficiency-induced increase of intracellular Fe, the enhanced release of catecholamines in Mg deficiency may be an additional mechanism of elevated MDA and LPO. Proof of this suggestion is the increase of MDA by isoproterenol in liver and heart (tab. 2).
The mechanism by which isoproterenol increased MDA may be oxygen free radicals, which are formed by its oxidation to o-quinone and adrenochrome [27].
Another mechanism may be local ischemia by isoproterenol. During ischemia xanthine dehydrogenase in endothelial cells is converted to xanthine oxidase, whereas hypoxanthine concentration is increased. This serves as substrate for xanthine oxidase. With beginning reperfusion oxygen free radicals are produced [28].
Moreover, in Mg deficiency the synthesis of prostaglandins, particularly of thromboxane A_2, which is combined with increased formation of oxygen radicals, was enhanced [29]. For detailed literature and discussion of the

Tab. 4: Concentration of Fe, Mg and Ca in plasma of rats fed various diets (I–VI) and s.c. injected with 3 mg/kg isoproterenol (Iso). See legend to tab. 2.

Group		n	Fe µmol/l	Mg mmol/l	Ca mmol/l
I,	-	4	38.8 ± 1.2	0.62 ± 0.01	2.53 ± 0.05
I,	Iso	4	12.6 ± 2.1[f]	0.73 ± 0.01[f]	2.45 ± 0.02
II,	-	4	36.0 ± 2.6	0.60 ± 0.01	2.55 ± 0.04
II,	Iso	6	11.3 ± 1.7[f]	0.76 ± 0.02[f]	2.38 ± 0.06[d]
III,	-	5	36.4 ± 3.0	0.62 ± 0.02	2.50 ± 0.03
III,	Iso	4	8.6 ± 1.4[f]	0.71 ± 0.02[e]	2.37 ± 0.03[d]
IV,	-	5	30.8 ± 3.1[a]	0.102 ± 0.007[c]	2.53 ± 0.01
IV,	Iso	8	8.7 ± 1.6[f]	0.133 ± 0.008[e]	2.41 ± 0.03[e]
V,	-	6	30.8 ± 3.0[a]	0.087 ± 0.003[c]	2.50 ± 0.02
V,	Iso	8	10.7 ± 1.7[f]	0.155 ± 0.013[f]	2.40 ± 0.04[d]
VI,	-	5	31.9 ± 2.0[a]	0.079 ± 0.005[c]	2.51 ± 0.04
VI,	Iso	5	12.8 ± 0.9[f]	0.125 ± 0.026	2.36 ± 0.05[d]

Tab. 5: Fe, Ca and Mg content in liver (mmol/kg dry weight). For legend see tab. 2.

Group		n	Fe	Ca	Mg
I,	-	4	3.57 ± 0.19	2.42 ± 0.06	27.7 ± 0.1
I,	Iso	4	4.44 ± 0.37	2.57 ± 0.03	32.9 ± 0.3[f]
II,	-	4	4.02 ± 0.60	2.41 ± 0.06	27.1 ± 0.5
II,	Iso	6	4.82 ± 0.41	2.42 ± 0.07	31.0 ± 1.0[e]
III,	-	5	4.13 ± 0.20	2.35 ± 0.11	27.1 ± 0.7
III,	Iso	4	3.89 ± 0.52	2.57 ± 0.10	29.6 ± 0.2[d]
IV,	-	5	10.4 ± 2.4[a]	2.58 ± 0.19	27.1 ± 1.1
IV,	Iso	8	12.7 ± 1.6	2.47 ± 0.10	33.4 ± 0.8[f]
V,	-	6	10.6 ± 1.6[b]	2.65 ± 0.12	25.9 ± 0.8
V,	Iso	7	12.8 ± 1.6	2.48 ± 0.12	34.6 ± 0.9[f]
VI,	-	5	11.0 ± 2.2[a]	2.50 ± 0.22	26.7 ± 1.2
VI,	Iso	5	11.2 ± 0.8	2.42 ± 0.11	33.8 ± 0.6[f]

pathobiochemical mechanisms see References [13, 14, 30].

The MDA levels in 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 [11, 31, 32]. Therefore, the formation of free radicals in Mg deficiency may be higher than indicated by MDA formation, because a part of MDA was metabolized and a part of free radicals was scavenged by vitamin E, leading to destruction of vitamin E in Mg deficiency (tab. 3).

Electrolyte concentrations

Plasma (tab. 4)

Mg deficiency caused a drastic reduction of plasma Mg and a small reduction of plasma Fe, as already described [33]. Vitamin E content of the diets had no effect on plasma concentrations of Fe, Mg and Ca.

Four hours after injection of isoproterenol we found a drastic reduction of plasma Fe, a significant increase in plasma Mg and a small reduction of plasma Ca.

The isoproterenol-induced reduction of plasma Fe may be caused by increased intracellular uptake. Since the amount of plasma Fe (transferrin-bound Fe) is extremely small compared to cellular Fe, an increased cellular Fe (transferrin) uptake cannot be verified. The increase in plasma Mg by isoproterenol is probably caused by release of Mg from hydroxyapatite of bone (in preparation).

Liver (tab. 5)

In Mg deficiency, Fe content of liver was drastically increased independent of vitamin E nutrition, as described and discussed in a preceding paper [33]. Isoproterenol had no significant effect on liver Fe.

Ca content of liver was unchanged by Mg deficiency, vitamin E or isoproterenol.

Mg content in liver was not significantly changed by Mg deficiency and vitamin E. However, 4 hours after injection of isoproterenol liver Mg was increased by about 20% independent of vitamin E.

Other investigators found a 28% and 22% increase in liver Mg 1 day and 2 days after s.c. injection of 65 mg/kg isoproterenol [34]. The biochemical mechanism was investigated in detail [34a].

Kidney (tab. 6)

Fe content of kidney was increased in Mg deficiency by about 35%, as found by other authors [35].

Ca content of kidney was increased by Mg deficiency which is one of the typical effects of Mg deficiency.

Remarkably, high vitamin E nutrition prevented the Mg deficiency-induced Ca deposition in the kidney. Probably a toxic cellular event (oxygen free radicals) which can be prevented by vitamin E is involved in Mg deficiency-

Tab. 6: Fe, Ca and Mg content of kidney (in mmol/kg dry weight). For legend see tab. 2.

Group	n	Fe	Ca	Mg
I, -	4	3.09 ± 0.20	4.35 ± 0.39	30.3 ± 2.1
I, Iso	4	2.61 ± 0.20	5.12 ± 0.59	33.6 ± 0.7
II, -	4	3.14 ± 0.16	4.43 ± 0.16	29.4 ± 2.2
II, Iso	6	2.87 ± 0.12	4.85 ± 0.08	31.4 ± 1.6
III, -	5	3.10 ± 0.13	4.48 ± 0.11	31.1 ± 0.6
III, Iso	4	2.59 ± 0.18	4.53 ± 0.19	30.4 ± 0.8
IV, -	5	4.26 ± 0.26^{b}	70.2 ± 27.7^{a}	29.5 ± 0.9
IV, Iso	8	3.62 ± 0.13	22.9 ± 8.3	29.1 ± 0.5
V, -	6	4.27 ± 0.25^{b}	32.8 ± 10.1^{a}	30.1 ± 0.8
V, Iso	7	3.69 ± 0.12	56.7 ± 19.9	30.9 ± 0.5
VI, -	5	4.09 ± 0.20^{b}	5.58 ± 1.02	29.8 ± 0.4
VI, Iso	5	3.58 ± 0.22	5.46 ± 0.50	30.3 ± 0.6

Tab. 7: Fe, Ca and Mg content of heart (in mmol/kg dry weight). For legend see tab. 2.

Group	n	Fe	Ca	Mg
I, -	4	4.07 ± 0.12	2.47 ± 0.49	35.2 ± 0.8
I, Iso	4	4.08 ± 0.16	5.55 ± 0.60^{e}	30.1 ± 1.2^{d}
II, -	4	4.00 ± 0.30	2.55 ± 0.15	34.3 ± 1.2
II, Iso	6	4.21 ± 0.14	5.42 ± 0.42^{f}	28.5 ± 1.1^{e}
III, -	5	4.11 ± 0.40	2.55 ± 0.17	34.0 ± 1.3
III, Iso	4	4.38 ± 0.23	3.00 ± 0.25	28.9 ± 1.5^{d}
IV, -	5	5.28 ± 0.30^{b}	2.96 ± 0.70	28.3 ± 1.0^{b}
IV, Iso	8	5.43 ± 0.21	6.04 ± 0.34^{e}	27.4 ± 1.5
V, -	6	5.02 ± 0.16^{b}	2.82 ± 0.19	29.8 ± 1.9^{a}
V, Iso	7	5.79 ± 0.07^{e}	5.69 ± 0.48^{f}	28.7 ± 0.6
VI, -	5	5.36 ± 0.20^{c}	3.03 ± 0.30	32.6 ± 0.6^{a}
VI, Iso	5	5.31 ± 0.23	4.19 ± 0.34^{d}	30.5 ± 1.3

induced kidney calcification. Mg content was not significantly affected by the treatments.

Heart (tab. 7)

In Mg deficiency, Fe content of heart was increased by 25%. Thus, Fe increase in heart was less than in kidney and much less than in liver.

Isoproterenol caused a small additional Fe increase in the hearts of Mg-deficient-vitamin E-reduced rats.

Mg content of the heart was reduced by Mg deficiency or isoproterenol, which is in agreement with the results of other authors [36]. Injection of isoproterenol to Mg-deficient rats did not further reduce Mg content. Vitamin E had no effect on Mg deficiency – or isoproterenol-induced reduction of cardiac Mg. Ca content of the heart was drastically increased by isoproterenol as found by other investigators [3, 36]. Vitamin E supplementation could almost prevent this effect. It could also protect against Mg-deficiency-induced cardiomyopathy in hamsters [15]. Therefore, it was suggested that oxygen free radicals may be involved in cardiotoxicity [15].

There is a well-known relationship between intracellular Ca and LPO caused by oxygen free radicals in hepatotoxicity [10].

In order to analyze the relationship between cardiotoxicity (measured by cardiac Ca) and LPO (measured by cardiac MDA) vitamin E and MDA (fig. 1) and MDA and Ca (fig. 2) were correlated.

Fig. 1 shows that MDA is negatively correlated to vitamin E up to 110 µmol/kg wet weight indicating that vitamin E can reduce LPO by scavenging oxygen free radicals. Isoproterenol caused the same increase in MDA independent of vitamin E content. However, at high vitamin E contents of the hearts, there was no significant increase in MDA, indicating that high vitamin E contents can almost completely prevent isoproterenol-induced LPO.

Correlation of LPO with cardiac Ca (fig. 2) showed that in groups I, II, IV and VI isoproterenol induced almost the same increase in cardiac Ca and MDA, which were drastically reduced in groups III and VI with high vitamin E supplementation (fig. 2). This result may indicate that isoproterenol-induced LPO is involved in isoproterenol-induced Ca uptake of the heart. However, this correlation is no proof of causality. At low cardiac vitamin E (isoproterenol-untreated rats of groups II and V), MDA was higher than in isoproterenol-treated rats of group I (fig. 1, 2) although these rats of groups II and V expressed lower cardiac Ca than isoproterenol-treated rats of group I (fig. 2).

This comparison shows, that there is no simple correlation between MDA and Ca.

Therefore, it may be alternatively suggested that isoproterenol-induced Ca uptake is mediated by a β-adrenergic

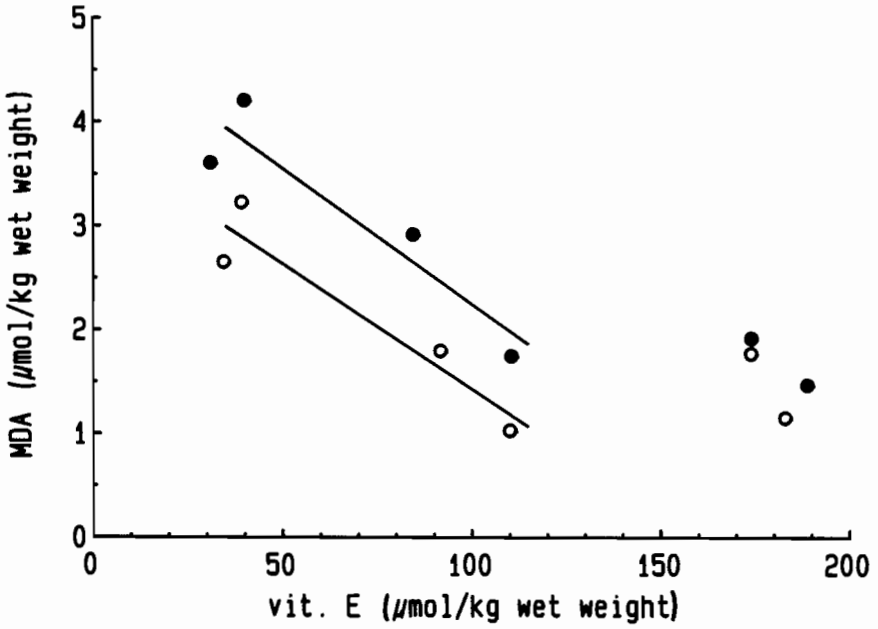

Fig. 1: Correlation of cardiac MDA (tab. 2) and cardiac vit. E (tab. 3); ○, without isoproterenol, ●, isoproterenol-injected rats.

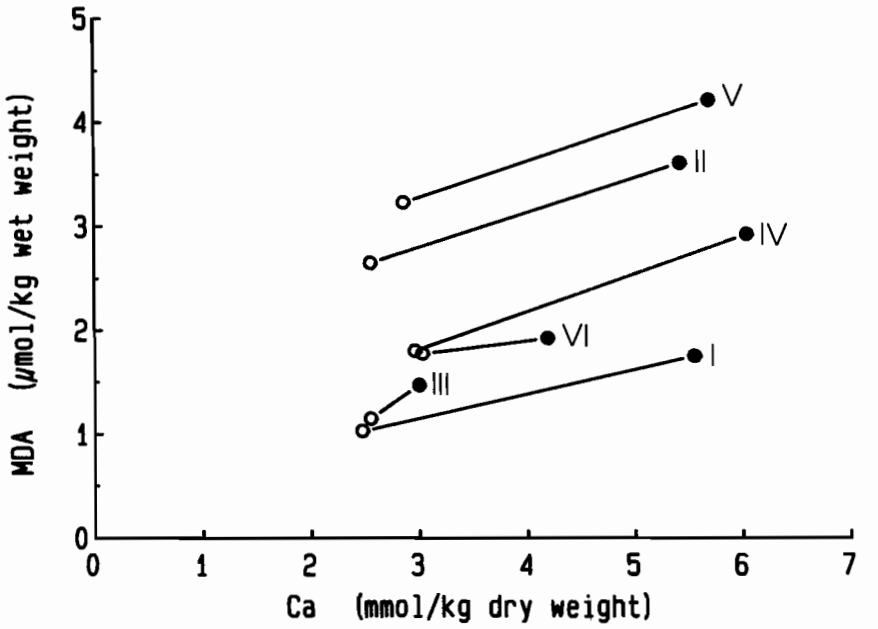

Fig. 2: Correlation of cardiac MDA (tab. 2) and cardiac Ca (tab. 7). ○, without isoproterenol, ●, isoproterenol-injected rats. I–VI, dietary group.

effect and high vitamin E content in the cell membrane may inhibit the β-adrenergic system or the β-adrenergic-stimulated Ca channel. In agreement with this conclusion it was shown that vitamin E can protect against Ca-induced cell injury independent of its antioxidant effects [37].

This action of vitamin E can also explain its cardioprotective effect in Mg deficiency-induced cardiomyopathy of hamsters [15].

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