Metabolic effects of a low-magnesium diet in pigs

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1. Pigs were fed on semi-purified food. The magnesium content of the experimental diet was 1·0 and that of the control diet 1·9 g/kg.

2. In the low-Mg group serum triglycerides and blood lactate values were increased and base excess and standard bicarbonate values were decreased, indicating metabolic acidosis.

3. A significant positive dependence was found between blood pH and serum Mg:Ca value as well as between blood pH and serum Mg, and also between body temperature and blood lactate values in the low-Mg group. None of these dependences was significant in the control group with magnesium acetate supplementation in the food.

4. No specific histopathological changes were found in heart, liver or kidney of the experimental animals.

5. The results indicate that an increase in serum triglycerides does not need to depend on the amount or quality of food fat.

6. The present study shows that, in pigs, a low-Mg diet may cause metabolic disturbances in instances when the food Mg content is distinctly higher than the normal values recommended by the (UK) Agricultural Research Council (1966) and (US) National Research Council (1968).

The two distinctive symptoms of magnesium deficiency across species are hyperirritability and metastatic calcifications (Jacobson *et al.* 1972). It is also known that Mg deficiency can cause many kinds of cardiovascular and gastrointestinal disorders (Szelényi, 1973; Seelig & Heggtveit, 1974).

According to the (UK) Agricultural Research Council (ARC; 1966) and (US) National Research Council (NRC; 1968) approximately 0·4 g Mg/kg pig food is a sufficient amount to satisfy requirements. The Mg content of ordinary pig food is 4- to 5-fold greater than this. On the basis of clinical experience of pigs it was proposed by Nuoranne (1971) that leg weakness and cannibalism, for example, could be successfully treated by means of abundant doses of magnesium oxide or sulphate.

Since 1972 all industrial pig foods in Finland have been supplemented by a considerable amount of Mg. In 1974 and 1978 indirect evidence was presented by Nuoranne (1974, 1978*b*) which further suggested that this Mg supplementation had exerted a favourable effect on many disorders like bruxism, splay leg, leg weakness, hepatosis dietetica, acute cardiac failure and anaemia. A great part of these disorders seemed to be functional failures of muscle tissue.

Table 1. *The composition of the food by analysis*

	g/kg
Dry matter	895
Crude protein (nitrogen × 6·25)	190
Crude fibre	32
Crude fat	17
Ash	44
N-free extract	612
Calcium	8·8
Phosphorus	5·3
Magnesium	1·0
Potassium	4·1
	mg/kg
Copper	110
Zinc	213
Iron	365

On the basis of changes in enzyme activities in serum it has been found that pigs fed on an ordinary diet may suffer from Mg deficiency despite the fact that the food Mg content is distinctly higher than normal values recommended by ARC (1966) and NRC (1968) (Nuoranne, 1978a).

In this study pigs were fed on a semi-purified diet with a low Mg content and an attempt was made to find out if this diet caused a state of Mg deficiency in the experimental pigs.

EXPERIMENTAL

Animals, food and the course of the experiment

The four experimental and the four control pigs originated from the same litter. On the basis of pre-experimental determinations, the experimental groups were made as equal as possible in terms of serum Mg, body-weight and sex.

The experimental food consisted of (g/kg) white wheat flour 550, barley flour 215, oat flour 100, dried blood 100. It was supplemented by (g/kg) dicalcium phosphate 20, limestone 6, and the vitamin and mineral mixture (excluding Mg) by which industrial pig foods are supplemented during preparation (mg/kg NaCl 3296·5, KJ 5, CuO 120, $CoSO_4 . 7H_2O$ 8, ZnO 170, $FeSO_4 . 7H_2O$ 300, MnO 100, sodium selenite 0·2, retinol 3000 μg/kg, ergocalciferol 50 μg/kg and α-tocopherol 30 mg/kg).

The food contents analysed are shown in Table 1. The calculated digestible protein content of the food was 164 g/kg. The food of the control group was daily supplemented with 8 g magnesium acetate/kg food. The calculated Mg content in the food of the control group was thereby raised to 1·9 g/kg.

The drinking-water of the pigs contained 5–6 mg Mg/l and there was no straw in the pens. Consequently, the gain of Mg from sources other than food was minimized.

After weaning the experimental pigs were fed on an ordinary pig diet (2·1 g Mg/kg). During the last week before the experiment pigs were stepwise accustomed to eat experimental food with 1·9 g Mg/kg.

At the beginning of the experiment the pigs were 7 weeks old. The average weight of the experimental pigs was 19·7 kg and that of control pigs 19·5 kg. During the 61 d experiment, the average gain of weight was 29·1 kg (477 g/d) in the experimental group and 30·7 kg (503 g/d) in the control group.

On the experimental days 0, 6, 9, 14, 22, 34, 43 and 61 blood samples were taken from vena cava cranialis at 07.00 hours (before feeding).

Biochemical determinations

Pyruvate and lactate were estimated from blood after perchloric acid precipitation using lactate dehydrogenase (*EC* 1.1.1.27) as an assay enzyme (Czok & Lamprecht, 1974; Gutman & Wahlefeld, 1974). Enzymic serum triglyceride assay after enzymic hydrolysis was employed for neutral fat determination (Wahlefeld, 1974). All three assays were performed using assay kits obtained from Boehringer Mannheim GmbH, Germany, and the samples were stored at $-20°$ before assay.

For the determination of acid–base balance, blood was taken from the ear veins into heparinized capillary tubes (Radiometer D 551/10/80; Radiometer, Copenhagen) and the acid–base measurements were made using an Astrup Radiometer Design 22 in a hospital laboratory.

Alanine aminotransferase (*EC* 2.6.1.2; ALAT), aspartate aminotransferase (*EC* 2.6.1.1; ASAT), lactate dehydrogenase and D-glutamyltransferase (*EC* 2.3.2.1; γ-GT) activities, glucose, creatinine and total protein in serum were determined by routine clinical methods using a Gilford 3500 computer-directed analyser (Gilford Instrument Laboratories Inc., Oberlin, Ohio). Mg and calcium levels in serum were determined by means of atomic absorption spectroscopy, serum inorganic phosphorus using Hitachi-Perkin-Elmer 139 UV-VIS spectrophotometer, and serum sodium using Corning flame photometer 430.

Histochemical and enzyme-histochemical methods

When the pigs were slaughtered, tissue samples were taken from heart, liver and kidney for light microscopy. Transmural samples of myocardium were cut from the anterior wall of each ventricle and the interventricular septum. Each sample was divided into two parts. Half was fixed in buffered formalin (100 ml/l) for 48 h, embedded in paraffin, cut at 5 μm and stained with haematoxylin–eosin. The other half was frozen in liquid nitrogen and 10 μm sections were cut on a cryostat and stained for β-hydroxybutyrate dehydrogenase (*EC* 1.1.1.30; Barka & Anderson, 1965).

Statistical methods and reference values

Two-way analysis of variance including repeated measures (split-plot design) was used for testing the differences between the groups, and linear regression analysis for testing the dependences between various measurements within the groups. In addition to serum Mg, Ca, the molar Mg:Ca value or lactate, time (*T*, d) was used as an independent variable in the regression equations.

The reference values of serum triglycerides, lactate and pyruvate are the average values of eight samples which were taken from randomly selected pigs aged 2 months and fed on an ordinary pig diet with 2·1 g Mg/kg food. For the reference values for acid–base measurements and serum Mg and Mg:Ca values two litters each with six pigs were grown from the age of 2 months to 3–3·5 months and blood sampling was carried out every 2 weeks. A total of forty-two samples were examined. Reference values (mean $\pm$ SE) were: serum Mg 0·88 $\pm$ 0·01 mM, serum Mg:Ca 0·333 $\pm$ 0·005, triglycerides 504 $\pm$ 12 mg/l, pyruvate 0·18 $\pm$ 0·01 mM, lactate 4·4 $\pm$ 0·1 mM, base excess 0·3 $\pm$ 0·4 mM, standard bicarbonate 24·6 $\pm$ 0·4 mM, actual pH 7·31 $\pm$ 0·01, partial pressure of carbon dioxide 58 $\pm$ 0·9 mmHg.

RESULTS

In the low-Mg group a statistically significant increase in serum triglycerides and lactate values, and a decrease in base excess and standard bicarbonate values were found (two-way analysis of variance, split-plot design; Table 2). The changes in serum triglycerides,

Table 2. *Serum magnesium (mM), Mg:Ca and triglycerides (mg/l), base excess (mM), standard bicarbonate (mM) and lactate (mM) in groups of four pigs fed on a low-magnesium diet or a control diet**

(Values are means with their standard errors)

	Serum Mg (mM)				Serum Mg:Ca				Serum triglycerides (mg/l)			
	Control group		Low-Mg group		Control group		Low-Mg group		Control group		Low-Mg group	
Day	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	0·89	0·04	0·86	0·03	0·335	0·011	0·320	0·017	491	29	502	24
6	0·78	0·02	0·79	0·05	0·280	0·012	0·277	0·012	500	22	511	16
9	0·75	0·01	0·74	0·02	0·273	0·003	0·273	0·006	492	16	514	31
14	0·85	0·02	0·83	0·03	0·320	0·009	0·309	0·010	505	16	544	23
22	0·79	0·02	0·77	0·02	0·293	0·012	0·287	0·023	510	12	580	14
34	0·90	0·03	0·83	0·02	0·342	0·011	0·307	0·011	501	18	538	22
43	0·82	0·02	0·78	0·03	0·312	0·010	0·306	0·008	507	28	574	31
61	0·84	0·03	0·81	0·04	0·308	0·007	0·304	0·002 $(P < 0.01)$†		510	16	572	34 $(P < 0.01)$†

	Base excess (mM)				Standard bicarbonate (mM)				Lactate (mM)			
	Control group		Low-Mg group		Control group		Low-Mg group		Control group		Low-Mg group	
Day	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	−1·0	0·3	−1·9	0·9	23·5	0·3	23·0	0·8	4·3	0·4	4·4	0·5
6	0·4	0·4	−0·4	0·8	24·8	0·3	24·0	0·7	4·3	0·5	4·7	0·4
9	−0·1	0·5	−0·3	0·7	24·3	0·4	24·2	0·6	4·2	0·4	4·8	0·2
14	−0·7	0·9	−0·9	0·6	24·5	0·6	23·6	0·5	4·3	0·4	4·9	0·5
22	0·6	1·0	−1·7	0·8	24·4	0·7	22·9	0·7	4·7	0·6	4·8	0·5
34	−1·9	0·6	−2·4	1·0	22·8	0·4	22·5	0·8	4·5	0·5	5·0	0·3
43	3·0	1·2	0·4	1·6	26·8	1·2	24·8	1·4	4·6	0·4	5·3	0·5
61	0·6	1·4	−5·4	0·8 $(P < 0.01)$†	24·7	1·1	20·2	0·6 $(P < 0.02)$†	4·6	0·5	6·3	0·2 $(P < 0.01)$†

* For details of diet, see Table 1 and p. 54.

† The statistical significance of differences between the groups (two-way analysis of variance including repeated measures, split-plot design).

No statistically significant difference between the groups was found in Ca, sodium, inorganic phosphorus, blood pH, partial pressure of carbon dioxide, pyruvate, alanine aminotransferase, aspartate aminotransferase, D-glutamyltransferase, creatinine or total protein in serum or in the body temperature.
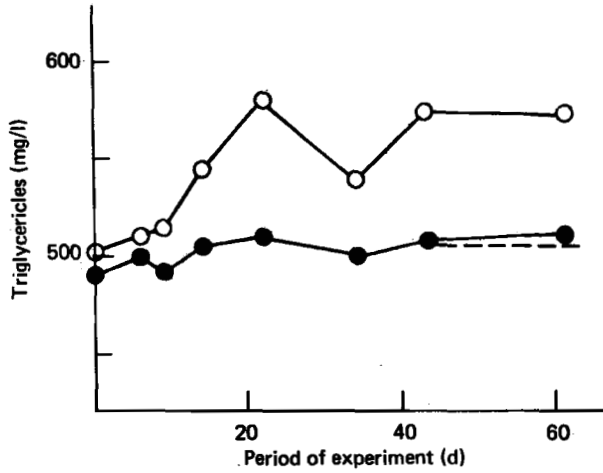
Fig. 1. Changes in serum triglycerides (mg/l) in groups of four pigs fed on a low-magnesium diet (○—○) or a control diet (●—●); (----) reference level. For details of diet, see Table 1 and p. 54.
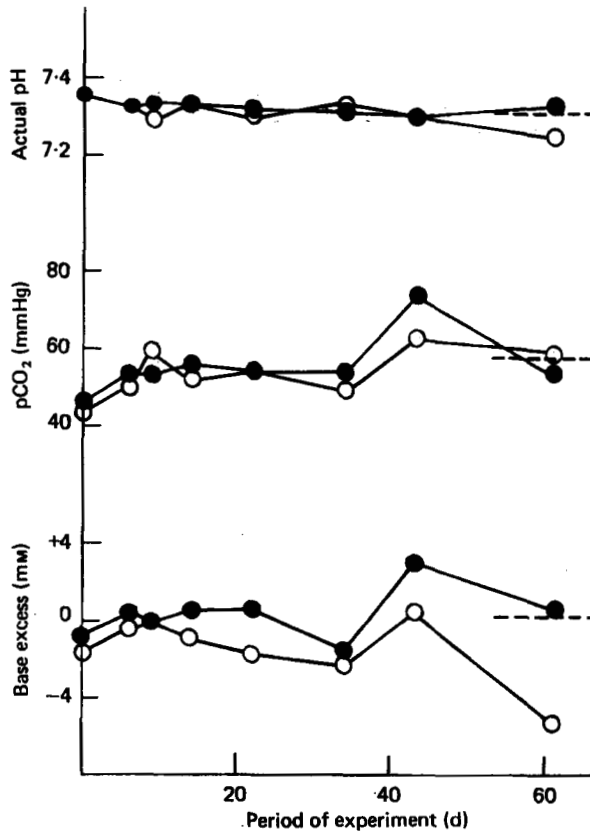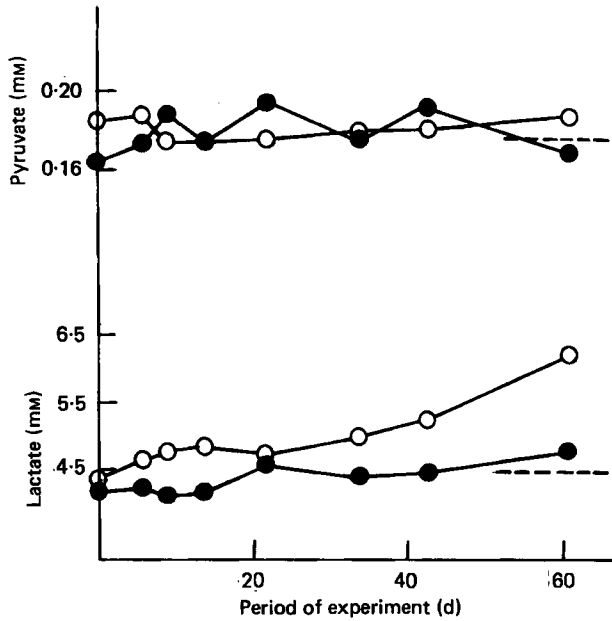

Fig. 2. Changes in blood actual pH, partial pressure of carbon dioxide (pCO₂; mmHg) and base excess (mM) in groups of four pigs fed on a low-magnesium diet (○—○) or a control diet (●—●); (----) reference level. For details of diet, see Table 1 and p. 54.

Fig. 3. Changes in blood lactate (mM) and pyruvate (mM) values in groups of four pigs fed on a low-magnesium diet (O—O) or a control diet (●—●); (---) reference level. For details of diet, see Table 1 and p. 54.

Table 3. *Dependences between the various measurements within the groups according to the linear regression analysis carried out on days 14–61.*

(Regression coefficients (c) from estimated equations of type $y = a + b \times$ period of experiment $(d) + cx$ with their level of statistical significance are given)

Independent variable ...	Control group			Low-magnesium group		
Dependent variable	Magnesium	Calcium	Mg:Ca	Magnesium	Calcium	Mg:Ca
Blood pH	—	—	—	0·29*	—	1·25**
Serum glucose	—	—	—	−11·34**	—	—
Serum inorganic phosphorus	2·20***	—	3·22*	—	—	—
ALAT	—	—	96·07*	—	—	—
γ-GT	—	−61·64*	303·17*	—	—	—
Creatinine	307·55*	—	—	—	73·72*	—
Total serum protein	113·40*	—	—	—	—	—

ALAT, alanine aminotransferase; γ-GT, D-glutamyltransferase.

* $P < 0.05$,   ** $P < 0.01$,   *** $P < 0.001$.

Astrup measurement, lactate and pyruvate are shown in Figs. 1–3 and in Table 2 in which serum Mg and molar Mg:Ca values are also given.

In the low-Mg group pH decreased with time (d) on days 14–61. A statistically significant positive dependence between blood pH and molar Mg:Ca in serum was also found as shown by the following estimated equation:

pH = 6·98 − 0·0017T + 1·25 Mg:Ca ($P < 0.001$, $P < 0.01$). The equation represents 62·0 % of the total variation of blood pH.

More information of dependences between various measurements is shown in Table 3. All information originates from statistically significant equations. For example, in the low-Mg group a statistically significant positive dependence was found between blood pH and serum Mg, and a negative one between serum glucose and Mg.

Furthermore, in the control group a statistically significant positive dependence was found between ALAT activity and molar Mg:Ca in serum as well as between γ-GT and Mg:Ca. A negative dependence was found between γ-GT activity and serum Ca.

In the low-Mg group, a statistically significant positive dependence was also found between body temperature and blood lactate content as shown by the following estimated equation:

$$\text{body temperature} = 38\cdot49 - 0\cdot00064T + 0\cdot017 \text{ *lactate content } (*P < 0\cdot05).$$

No specific histopathological change was found in heart, liver or kidney of the experimental animals.

DISCUSSION

The Mg content of the experimental diet, $1\cdot0$ g/kg, was approximately half that in ordinary pig food. This was achieved by replacing oat flour mainly by white wheat flour and by using dried blood as a protein source. Consequently, pigs were fed on a low-Mg diet instead of the so-called zero diet.

In both groups the phosphorus content of the food was abnormally low, and hence the Ca:P value relatively high ($1\cdot66$). However, this seems not to have disturbed the course of the experiment, because the important factors indicating metabolic disturbances in the low-Mg group were maintained close to the reference levels in the control group.

The use of venous blood in the determination of the Astrup measurements causes an increase in pCO_2 and a lowering in pH when compared with an ordinary determination from the arterial or capillary blood. Venous blood was used for practical reasons concerning sampling.

The maintenance of the triglyceride, base excess, standard bicarbonate and lactate values in the control group on the level of the reference values, and a statistically significant deviation from the control group values in all these measurements of the low-Mg group, indicate that the pigs of the low-Mg group very likely suffered from Mg deficiency. The minimum Mg content needed in the pig food according to the normal values recommended by the ARC (1966) and NRC (1968) seems, therefore, to be too low, as suggested by Nuoranne (1971, 1978*a, b*).

In earlier studies both increases and no changes in neutral fat concentration have been found in Mg-deficient animals (see Seelig & Heggtveit, 1974). A significant increase in triglycerides was found in the low-Mg group of the present work. The fat content of the experimental food was 17 g/kg only, thus indicating that the increase in serum triglycerides does not need to depend on the amount or quality of food fat. As the growth of the experimental pigs was rather good, the possibility that the increase in triglycerides was due to the mobilization of body fat is also unlikely.

It seems possible that the increase in serum triglycerides was secondary to changes in carbohydrate metabolism. This is suggested by the rises in blood lactate as well as in the lactic acid/pyruvic acid value in the low-Mg group. A possible explanation for these changes and for the lowering of the base excess and standard bicarbonate values of the low-Mg group might be a disturbance in oxidative phosphorylation. In Mg-deficient *Escherichia coli* an increased production of lactate and a decrease in respiration were

found (Günther & Mariss, 1968). In bacteria, Mg deficiency causes an increase in phospho-lipids (Günther et al. 1975).

Further studies are required to explain the mechanism by which the low-Mg diet induced the metabolic disturbances. Minor differences in serum Mg and Mg:Ca values between the groups are in agreement with the earlier finding that serum Mg cannot be considered as a reliable index of body Mg status (Nuoranne, 1978c).

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