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ORIGINAL CONTRIBUTION

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Dietary thiamin supply during lactation influences thiamin status in lactating rats and their offspring and the thiamin level in milk

Die Thiaminversorgung während der Laktation beeinflußt den Thiaminstatus von laktierenden Ratten und deren Nachkommen sowie den Thiaminspiegel der Milch

Summary This study was conducted to examine the effect of dietary thiamin, ranging from deficient to excessive supplies, on thiamin status of lactating rats and their offspring, and the thiamin level in milk. Therefore, after parturition, rat dams were divided into eight groups of 10 each, and were fed diets with 0, 2, 4, 6, 7, 40, 350 and 3 500 mg/kg thiamin over a total of 13 days during lactation. Milk for determining the thiamin concentration was obtained from day 6 and 13 of lactation. At day 14 of lactation rat dams and their offspring were used to ascertain the thiamin status including transketolase activity of blood, liver and brain, and thiamin concentration in body.

Thiamin supplies ranging from deficient to excessive dietary concentrations influenced both the thiamin levels of the lactating dams and their offspring within 13 days. Lactating rat dams fed a thiamin-free diet and their offspring were classified as thiamin-deficient on the basis of growth retardation and a lower activity of transketolase in blood, liver and brain. Within these variables transketolase in blood has been shown to be most sensitive, and reached a plateau feeding 6 mg/kg thiamin. The concentration of thiamin in milk ranged between 0.1 and 19 mg/kg. The findings also show that dietary thiamin had the strongest effect on thiamin in milk obtained from day 6 and 13 of lactation, and a deficient or suboptimal supply with thiamin was therefore not compensated for an intensified transfer of reserved body thiamin into milk. Also thiamin levels in tissues and carcass, which did not show any clear-cut saturation characteristic, increased with increasing dietary thiamin, and this dose-dependence was more marked in blood and liver than in carcass.

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Zusammenfassung Ziel der vorliegenden Studie war es, die Wirkung variierender Thiaminzulagen (Mangel bis Überschuß) in der Diät auf den Thiaminstatus laktierender Ratten und deren Nachkommen sowie auf den Thiaminspiegel der Milch zu untersuchen. Dazu wurden Ratten nach dem Werfen in 8 Gruppen (à 10 Tiere) eingeteilt, die während der Laktation über einen Zeitraum von 13 Tagen eine Diät mit 0, 2, 4, 6, 7, 40, 350 und 3 500 mg Thiamin/kg erhielten. Milch für die Bestimmung der Thiaminkonzentration wurde am 6. und 13. Laktationstag gewonnen. Am 14. Laktationstag wurden von allen Muttertieren und Würfen zur Ermittlung des Thiaminstatus die Aktivität der Transketolase in Blut, Leber und Gehirn sowie die Thiaminkonzentrationen im Körper gemessen.

Die variierenden Thiaminzulagen beeinflußten innerhalb von 13 Tagen sowohl den Thiaminstatus der laktierenden Ratten als auch den der Nachkommen. Thiamin-frei ernährte Ratten zeigten Thiaminmangelsymptome auf der Basis von Wachstumsminderung und erniedrigten Transketolaseaktivitäten in Blut, Leber und Gehirn. Dabei reagierte die Transketolase im Blut sensibler auf eine Thiaminunterversorgung als in Geweben und erreichte ein Plateau bei einer Zufuhr von 6 mg/kg Thia-

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min. Die Thiaminkonzentration in der Milch lag in einem Bereich zwischen 0,1 und 19 mg/kg. Verglichen mit anderen Geweben reagierte die Milch beider Laktationsabschnitte am stärksten auf eine Thiaminunter- bzw. -überversorgung. Darüber hinaus konnte eine fehlende oder suboptimale Thiaminversorgung nicht durch einen verstärkten Übertritt von Thiaminreserven aus dem Körper in die Milch kompensiert werden. Auch die Thiaminspiegel in den Geweben und im Restkörper erhöhten sich mit steigender Thiaminzufuhr, wobei sich die Dosisabhängigkeit, die jedoch kein eindeutiges Sättigungsverhalten zeigte, in Blut und Leber stärker widerspiegelte als im Restkörper.

Key words Dietary thiamin supply – lactation – thiamin status – milk – rat

Schlüsselwörter
Thiaminversorgung – Laktation – Thiaminstatus – Milch – Ratte

Introduction

The effects of dietary thiamin supplementation during gestation on thiamin status, performance and reproduction have been sufficiently researched in a series of experiments with rats (7, 12, 20). The data indicate that the thiamin needs of the rat dams are increased during pregnancy by growth of the placentae and the fetuses, and by the increments in the maternal stores (1). No attempt has been made to follow the thiamin status throughout the whole lactation cycle, the thiamin level in milk or to study the relationship between the biochemical status in offspring and the dietary intake of vitamin B_1 with the milk. This study was designed to examine the effects of dietary vitamin B_1 ranging from deficient to excessive supplies, which were fed during lactation on vitamin B_1 status of lactating rat dams and their offspring. The used thiamin supplementations from 0 to 7 mg/kg therefore comprised a deficient, a suboptimal, and a sufficient thiamin supply. Excessive thiamin supplementations of 40 and 350 mg/kg were chosen to mimic closely human situation during vitamin therapy. An extremely excessive supplementation of dietary thiamin in a concentration of 3 500 mg/kg was used to test whether there are saturation limits of maternal thiamin stores or untoward effects on suckling pups. The most widely accepted approach to evaluate thiamin nutriture is provided, at present, by the functional test based on the measurement of the transketolase activity (6). In body, thiamin acts after phosphorylation to thiamin diphosphate, as coenzyme of a number of enzymes, e.g. transketolase, alpha ketoglutarate dehydrogenase and pyruvate dehydrogenase. Since thiamin deficiency is seen particularly in organs with active metabolism, transketolase activity was besides blood measured in liver and brain. Since transketolase activity is not suitable for examining an excessive vitamin B_1 supply, which has been shown to interfere with lactation (11), assays of the thiamin concentrations in maternal tissues and milk, and in tissues of the suckling rats were made, additionally.

Material and methods

Animals and diets

In this experiment 80 female SPF Sprague-Dawley rats with an average body weight of 216.6 g ± 19.5 g were mated and fed a diet with 3 mg thiamin/kg during gestation. To equalize the vitamin B_1 status of the rats at the beginning of the experiment, rats received before pairing a diet with 3 mg thiamin/kg over 2 weeks. To guarantee an equal thiamin supply during gestation the food intake was restricted to 14.4 g/d. After parturition the dams were divided into 8 groups of 10 each and were fed diets supplemented with 0, 2, 4, 6, 7, 40, 350 and 3 500 mg thiamin/kg as thiamin · HCl. The analyzed thiamin concentrations were 0, 1.9, 4.0, 5.9, 6.8, 37, 347 and 3 487 mg/kg dry matter. During lactation the dams received the diets ad libitum. All the other components of the diet remained constant. All diets were fortified with recommended amounts of vitamins and minerals according to NRC (10). The composition of the basal semisynthetic diet is given in Table 1. Rats were housed individually in a controlled environment, in Macrolon cages in a room maintained at 23° C with a humidity of 55 %. All rats were kept under conditions of controlled lighting with a daily 12-h light:dark cycle, and had free access to drinking water.

At day 6 and day 13 of lactation dams were milked to obtain milk for determining the thiamin concentration. Therefore, the rat dams were anesthetized lightly with an intramuscular injection of Ketamine (0.2 ml/100 g) and Rompun (0.01 ml/100 g). Oxytocin (0.1 ml) was injected subcutaneously to stimulate milk flow. Oxytocin treatment was taken to have little impact on milk composition (9). Milk samples were collected from each nipple by gentle palpation using a sucking apparatus. All milk samples were obtained after only a 1-h-separation of dam and litter. At the end of the experimental period at day 14 of lactation the rat dams and their offspring were killed by decapitation after a light anesthesia with diethyl ether. Blood for determining the activity of transketolase was collected into heparin-coated vials. Liver samples were excised and stored at -80° C until analyzed for their

Table 1 Composition of the basal diet[1)]

Ingredient	Amount (g/kg)
Casein	200
Cornstarch	350
Sucrose	269
Coconut oil	77
Soybean oil	10
Fiber (cellulose)	30
Mineral mixture[2)]	42
Vitamin mixture[3)]	20
DL-Methionine	2

[1)] Basal diet analyzed had 0 mg/kg thiamin.

[2)] Minerals per kg diet: 13.60 g $CaCO_3$; 10.74 g $Na_2HPO_4 \cdot 2\ H_2O$; 8.20 g KH_2PO_4; 6.00 g KCl; 3.40 g $MgCl_2 \cdot 6\ H_2O$; 248.8 mg $FeSO_4 \cdot 7\ H_2O$; 219.9 mg $ZnSO_4 \cdot 7\ H_2O$; 123.1 mg $MnSO_4 \cdot H_2O$; 47.2 mg $CuSO_4 \cdot 5\ H_2O$; 9.0 mg KI; 4.5 mg $NiSO_4 \cdot 6\ H_2O$; 1.5 mg $NaSiO_3 \cdot 5\ H_2O$; 1.2 mg NaF; 667.0 µg $Na_2SeO_3 \cdot 5\ H_2O$; 570.0 µg $SnCl_2 \cdot 2\ H_2O$; 513.0 µg $CrCl_3 \cdot 6\ H_2O$; 504.0 µg $Na_2MoO_4 \cdot 2\ H2O$; 230.0 µg NH_4VO_3; sucrose to 42 g.

[3)] Vitamins per kg diet: 1.66 mg all-trans retinol; 25 µg cholecalciferol; 150 mg all-rac-α-tocopherol; 5 mg menadione sodium bisulfite; 10 mg riboflavin, 6 mg pyridoxine·HCl; 50 mg Ca-pantothenate; 20 mg nicotinic acid; 1 g choline chloride; 1 mg folic acid; 50 µg cyanocobalamin; sucrose to 20 g.

thiamin concentration and transketolase activity. Moreover, offspring were analyzed for their transketolase activity in brain and their thiamin concentration in blood. After removing the intestine from the body, the carcasses of dams and their offspring were used for the measurement of thiamin concentration. All samples obtained from rat pups within a litter were pooled.

Analytical procedures

Thiamin concentrations in blood and tissues were assayed by a modified thiochrome method of Rettenmaier et al. (15). The method was based on the conversion of the vitamer to the corresponding high fluorescent thiochrome with $K_3[Fe(CN)_6]$ after breaking down the organic matrix with H_2SO_4, and after enzymatic hydrolysis to release vitamin B_1 from the tissue with Taka-Diastase (Serva, Heidelberg, Germany) and purification using chromatography with a acidic cation exchanger (Amberlite CG 40, Serva). Determination of the fluorescent thiochrome was done with a spectrofluorometer (RF 5000, Shimadzu, Duisburg). For measuring thiochrome, the excitation wavelength was set at 378 nm and the emission wavelength at 430 nm.

The activity of transketolase in blood was measured using a micromethod of Warnock (22), and transketolase activity in tissues was determined as described by Brin (4). For both methods the coefficient of variation was below 10 %. For measuring transketolase in blood ribose-5-phosphate was added to the blood hemolysate, and incubated at 37° C for 5, 10 and 65 min, respectively, before stopping the reaction with trichloroacetic acid. The rate of formation of sedoheptulose-7-phosphate from ribose-5-phosphate assessed spectrophotometrically, reflects transketolase activity and thus can be expressed in terms of µkat/ml blood (µmol/s/ml blood).

Transketolase activity in tissue was determined spectrophotometrically by the macromethod of Brin (4). The rate of formation of sedoheptulose-7-phosphate from ribose-5-phosphate reflects the transketolase activity. The transketolase activity was expressed as µkat/g tissue and µmol of formed sedoheptulose-7-phosphate/s/g tissue, respectively.

Statistical analysis

The effect of dietary thiamin concentrations was compared for statistical significance ($p < 0.05$) using the Fisher-test. All data in this text are expressed as means ± standard deviations of the individual values. Statistical different means within a column are marked with different superscripts. Data of analyzed milk were treated by ANOVA with the factors dietary thiamin concentration, time of collection, and their interaction.

Table 2 Daily food intake and body weight at day 14 of lactation of rat dams and their offspring receiving different dietary thiamin concentrations[1),2)]

Group	Rat dams		Rat offspring	
	Daily food intake (g/d)	Body weight (g)	Body weight of litter (g)	Litter size (pups/litter)
0	16.6 ± 5.01[a]	207.3 ± 14.7[a]	143.3 ± 17.3[a]	10.1 ± 1.0
2	27.2 ± 4.02[bc]	254.3 ± 17.3[cd]	193.1 ± 24.4[bc]	10.0 ± 0.9
4	28.9 ± 4.51[bc]	251.1 ± 19.4[bcd]	210.2 ± 22.8[c]	10.1 ± 1.0
6	30.0 ± 4.41[c]	257.9 ± 16.6[cd]	193.9 ± 20.9[bc]	10.1 ± 0.6
7	26.4 ± 5.66[b]	262.8 ± 16.7[cd]	181.4 ± 19.9[b]	10.4 ± 1.2
40	27.8 ± 5.41[bc]	264.6 ± 15.6[d]	190.3 ± 11.2[b]	10.5 ± 1.2
350	27.2 ± 5.91[bc]	239.1 ± 17.1[b]	183.2 ± 20.1[b]	10.0 ± 0.9
3 500	27.5 ± 3.80[bc]	248.8 ± 15.8[bc]	190.2 ± 13.4[b]	10.2 ± 0.9

[1)] Data are means ± SD, n = 10 for each group.

[2)] Means in each column were compared by Fisher test; significantly different means are marked with superscripts ($p < 0.05$).

Results

At day 14 of lactation rat dams without any thiamin supply during lactation and their 13-day old offspring had a significantly lower feed intake and body weight than rats receiving thiamin with the diet (Table 2). A dietary thiamin concentration of already 2 mg/kg prevented growth retardation within 2 weeks in dams and their offspring. Although feed intake did not differ between rats fed suboptimal and excessive thiamin, dams supplemented with 350 and 3 500 mg thiamin/kg, respectively had a somewhat lower body weight than dams fed thiamin ranging from 2 to 40 mg/kg. However, the offspring of these dams did not show any weight gain differences, and also the amount of pups per litter was not affected by the various thiamin supplies.

At the end of the experimental period at day 14 of lactation rat dams without any thiamin supply were classified as thiamin-deficient on the basis of a significantly lower activity of transketolase in blood compared to the other groups (Table 3). This was obvious by a significantly lower utilization of ribose-5-phosphate and a significantly lower formation of sedoheptulose-7-phosphate. Also suboptimal thiamin supplies of 2 and 4 mg/kg led to a significantly lower utilization of pentose and formation of sedoheptulose in dams' blood relative to groups whose thiamin supplies ranged between 6 and 350 mg/kg. Rat dams receiving an excessive thiamin supply of 3 500 mg/kg showed the highest pentose utilization and sedoheptulose formation in blood. The dietary thiamin status of rat dams was also reflected in the 13-day-old offspring. Offspring derived from rat dams fed a thiamin-free diet had the lowest transketolase activity in blood. With increasing thiamin concentration in the dams' diet the utilization of pentose and formation of sedoheptulose in blood from offspring increased continuously and reached already a plateau feeding 6 mg/kg thiamin.

Lactating rats which were fed a thiamin-free diet had a significantly lower transketolase activity than rats from all the other groups. Also rats receiving 2 mg/kg thiamin had a somewhat lower transketolase activity than thiamin-adequate rats. Offspring from rat dams, which received a thiamin-free diet or a diet with 2 mg/kg thiamin had a significantly lower activity of transketolase in liver than offspring from rats fed adequate or excessive thiamin (Table 4). Maximum hepatic transketolase activity in offspring was reached, feeding rat dams with 4 mg/kg thiamin or more. In brain the transketolase activity was significantly lower in rat offspring deriving from dams without any thiamin supplementation relative to the other groups. A maximum enzyme activity in offspring was already reached in groups whose dams received 2 mg/kg or more thiamin with the diet.

At the 6th day of lactation from each animal about 3.3 g milk could be obtained for determination of thiamin concentration. At day 13 of lactation, the milk yield from thiamin-deficient rat dams was significantly lower than those from the other groups (1.8 ± 0.7 g vs. 4.0 ± 1.3 g). The thiamin concentration in milk obtained from the 13th day of lactation was higher than in milk from the 6th day (Table 5). The thiamin levels in milk from both qualifying dates were significantly influenced by the dietary thiamin supply (Table 5). In milk from both lactation periods an increase in dietary thiamin concentration led to a continuous elevation of the thiamin level in milk. Dams receiving 3 500 mg thiamin/kg had a 100-fold higher thiamin concentration in milk than dams fed a thiamin-free diet. Also thiamin levels in liver and carcass of the lactating dams significantly increased with increasing dietary thiamin concentrations. This dose-dependence was more marked in liver than in carcass. Also in rat offspring thiamin levels in blood, liver and carcass increased significantly with an elevating thiamin supply of the dams (Table 6), and the dose-response-relationship was closer in blood and liver than in carcass.

Table 3 Activity of transketolase in blood of lactating rat dams and their offspring receiving different dietary thiamin concentrations[1),2)]

Group	Activity of transketolase in blood (μkat/l)			
	Rat dams		Rat offspring	
	R-5P[3)] utilization	S-7P formation	R-5P utilization	S-7P formation
0	1.10 ± 0.21[a]	0.18 ± 0.09[a]	2.16 ± 0.46[a]	0.30 ± 0.14[a]
2	2.37 ± 0.51[b]	0.47 ± 0.09[b]	2.97 ± 0.43[b]	0.44 ± 0.12[b]
4	2.79 ± 0.35[b]	0.61 ± 0.17[c]	3.90 ± 0.48[c]	0.62 ± 0.08[c]
6	3.26 ± 0.59[c]	0.72 ± 0.10[d]	4.80 ± 0.41[de]	0.63 ± 0.14[c]
7	3.32 ± 0.65[cd]	0.74 ± 0.15[d]	5.15 ± 0.42[ef]	0.61 ± 0.08[c]
40	3.46 ± 0.39[cd]	0.74 ± 0.08[d]	4.96 ± 0.31[def]	0.61 ± 0.09[c]
350	3.28 ± 0.55[c]	0.78 ± 0.08[de]	4.64 ± 0.44[d]	0.75 ± 0.14[d]
3 500	3.74 ± 0.37[d]	0.84 ± 0.12[e]	5.22 ± 0.36[f]	0.68 ± 0.08[cd]

[1)] Data are means ± SD, n = 10 for each group.
[2)] Means in each column were compared by Fisher test; significantly different means are marked with superscripts ($p < 0.05$).
[3)] Abbreviations used: R-5P = ribose-5-phosphate; S-7P = sedoheptulose-7-phosphate.

Table 4 Activity of transketolase in liver of lactating rats and their offspring and transketolase activity in brain of rat offspring from lactating rat dams receiving different dietary thiamin concentrations[1,2]

Group	Activity of transketolase (μkat/kg)		
	Rat dams	Rat offspring	
	Liver (μkat/kg)	Liver (μkat/kg)	Brain (μkat/kg)
0	32.5 ± 6.5[a]	29.2 ± 2.0[a]	9.34 ± 0.7[a]
2	49.5 ± 7.2[b]	27.5 ± 4.5[a]	11.3 ± 2.7[b]
4	55.3 ± 6.7[bc]	38.5 ± 5.2[b]	13.0 ± 0.8[c]
6	56.5 ± 6.0[c]	39.8 ± 4.5[b]	11.5 ± 0.5[b]
7	55.8 ± 6.7[c]	38.0 ± 7.0[b]	12.0 ± 0.3[b]
40	54.7 ± 6.8[bc]	27.8 ± 1.5[a]	11.5 ± 0.3[b]
350	55.8 ± 4.3[c]	37.7 ± 1.5[b]	11.8 ± 0.3[b]
3 500	56.3 ± 8.2[c]	38.8 ± 7.0[b]	11.7 ± 0.5[b]

[1] Data are means ± SD, n = 10 for each group.
[2] Means in each column were compared by Fisher test; significantly different means are marked with superscripts ($p < 0.05$).

Discussion

Dietary thiamin concentrations, ranging from deficient to excessive supplies, which were administered during lactation have been shown to vary the corresponding vitamin levels in body of rat dams and their offspring and have been shown to markedly influence the thiamin level in milk. Such a dose-response-relationship has been already found for other B vitamins including pyridoxin and riboflavin (2, 13, 14, 16, 18, 19). The current results also revealed that blood and liver react much stronger on the various dietary thiamin supplies than carcass, which previously has been also found for vitamin B_6 (20). Moreover, this study shows clearly that dietary thiamin had the strongest effect on thiamin in milk relative to other tissues or body fluids. Therefore, it can be established that milk is the best indicator representing the current dietary thiamin supply, whereas for both the liver and carcass a less marked dose-dependence does occur. The same could be observed in studies with the vitamins B_6 and B_2 (3, 16, 17) showing that milk reflects the corresponding vitamin level best. Therefore, it may be supposed that this finding applies to all water-soluble vitamins. However, in all cases the dose-dependence between dietary and body or milk thiamin did not show any clear-cut saturation characteristic. The strong variation of thiamin level in milk caused by the dose-response relationship between dietary thiamin and thiamin in milk, therefore caused marked differences in thiamin status of the rat pups, in which blood and liver reflected the thiamin level better than carcass.

The current results indicate clearly that 2 mg thiamin/kg may have been borderline for maximal food intake and growth. However, body weight gain or urinary levels of the corresponding micronutrient, which were often used to evaluate nutritional status have lacked specificity or been influenced by recent dietary intake or renal function. In contrast, the transketolase-activation test has been shown to be a more specific and sensitive measure of the vitamin B_1 status (5, 21). Also the determination of transketolase activity can be used as a tool to gain information on the sizes of body stores and hence on the thiamin long-term nutritional status. In the present study thiamin-free diets and diets with a suboptimal thiamin supply of 2 mg/kg caused a reduction in utilization of pentose and formation of sedoheptulose and a reduction in transketolase activity, respectively, which were indicative of an insufficient thiamin supply. According to this measurement the dose-response-relationship could be seen more clearly in blood than in liver or brain. According to the ascertained variables including the activity of transketolase and the thiamin concentration in body, it must be established that at least 6 mg/kg thiamin are required for lactating rats and their offspring. This is in accordance with the findings of Rajtek et al. (12) and

Table 5 Concentration of thiamin in milk at day 6 and 13 of lactation, liver and carcass of lactating rat dams receiving different dietary thiamin concentrations[1),2)]

Group	Thiamin concentration (mg/kg)			
	Milk		Liver	Carcass
	Day 6	Day 13		
0	0.09 ± 0.04[a]	0.10 ± 0.05[a]	0.94 ± 0.15[a]	0.44 ± 0.08[a]
2	0.39 ± 0.10[ab]	0.47 ± 0.07[ab]	2.65 ± 0.37[b]	1.03 ± 0.10[b]
4	0.89 ± 0.34[abc]	1.47 ± 0.43[abc]	4.48 ± 0.33[c]	1.55 ± 0.28[c]
6	1.60 ± 0.28[abc]	2.49 ± 0.21[bcd]	6.94 ± 1.20[d]	2.07 ± 0.14[d]
7	1.59 ± 0.17[abc]	2.80 ± 0.33[cd]	7.69 ± 0.80[d]	2.16 ± 0.17[d]
40	2.77 ± 0.41[cd]	5.28 ± 1.09[e]	9.36 ± 0.84[e]	2.81 ± 0.27[e]
350	4.38 ± 0.72[de]	8.02 ± 1.30[f]	12.18 ± 1.13[f]	3.78 ± 0.38[f]
3 500	9.70 ± 1.08[f]	18.90 ± 9.56[g]	16.79 ± 1.91[g]	–[3)]

[1] Data are means ± SD, n = 10 for each group.
[2] Means in each column were compared by Fisher test; significantly different means are marked with superscript ($p < 0.05$).
[3] not analyzed.

Table 6 Concentration of thiamin in blood, liver and carcass of rat offspring from lactating rat dams receiving different dietary thiamin concentrations[1),2)]

Group	Thiamin concentration		
	Blood (µg/100 g)	Liver (mg/kg)	Carcass (mg/kg)
0	1.56 ± 0.08[a]	1.20 ± 0.14[a]	0.34 ± 0.03[a]
2	3.19 ± 0.18[b]	2.42 ± 0.40[b]	0.52 ± 0.04[b]
4	5.18 ± 0.19[c]	7.71 ± 0.47[c]	1.19 ± 0.10[c]
6	8.52 ± 0.25[d]	11.92 ± 0.39[d]	1.55 ± 0.05[d]
7	11.47 ± 0.44[e]	13.00 ± 0.73[e]	1.81 ± 0.08[e]
40	14.66 ± 1.33[f]	15.14 ± 0.63[f]	2.22 ± 0.15[f]
350	17.04 ± 0.48[g]	16.43 ± 0.64[g]	2.40 ± 0.05[g]
3 500	23.31 ± 1.57[h]	17.48 ± 0.38[h]	3.37 ± 0.39[h]

[1)] Data are means ± SD, n = 10 for each group.
[2)] Means in each column were compared by Fisher test; significantly different means are marked with superscripts ($p < 0.05$).

Roth-Maier et al. (20) showing that the thiamin requirement for growing and pregnant rats ranges between 4 and 8 mg/kg. Although the determination of the transketolase activity is a very sensitive parameter for the registration of deficient or suboptimal supply, the assay of thiamin concentration in tissues and body fluids is more suitable for the determination of both a deficient and an excessive supply.

Since the vitamin status in organism changes very fast with the dietary supply, it is understandable that an adequate thiamin supply during gestation cannot compensate a lack of thiamin during lactation for both the maternal organism and the offspring. Also a suboptimal supply with thiamin during lactation therefore was not compensated for an intensified transfer of thiamin into milk, in order to protect offspring from thiamin deficiency symptoms. From the current results it can be concluded that an homeorhetic control mechanism, including the stabilization of the corresponding micronutrient concentration in milk over a wide range of dietary supply, which is true of trace elements (8), does not exist for thiamin. Therefore, an adequate thiamin supply during lactation is very important to provide the offspring with adequate thiamin.

In conclusion the present findings show that a varying thiamin supply during lactation ranging from deficient to excessive dietary levels influenced both the thiamin levels of the lactating rat dams and their offspring and markedly varied the thiamin level in milk. These alterations have been shown to occur within a relatively short time of 13 days. The most sensitive variables for determining thiamin status are the transketolase activity in blood and the thiamin concentration in milk.

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