

Effect of cow's milk factors on the bioavailability of non-milk thiamine in rats

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Summary — The influence of milk factors on the bioavailability of non-milk thiamine was assessed using thiamine-deficient young rats. Twenty-eight h dialysed raw cow's milk (3.5% fat) was used as the source of milk factors. Repletion test diets provided were (A) dialysed milk alone (59 µg) bound thiamine, (B) dialysed milk (59 µg) plus pure thiamine (256 µg) or (C) pure thiamine (315 µg) as thiamine source, for 21 d. Total hepatic thiamine content, erythrocyte transketolase (ETK) activity and TPP (thiamine pyrophosphate) effect on ETK activity were selected as response criteria. Group B receiving dialysed milk supplemented with pure thiamine had significantly ($P < 0.05$) greater total hepatic thiamine content and ETK activity restoration than the other groups. Likewise, the TPP effect was lowest ($19 \pm 0.34\%$) in group B as compared to the other groups. The estimate of the influence of milk factors on the bioavailability of non-milk thiamine from other dietary sources after correcting for baseline data on total hepatic thiamine content and ETK activity restoration showed that bioavailability of non-milk thiamine was enhanced by > 2 -fold in the presence of milk factors.

milk factor / thiamine / bioavailability

Résumé — Rôle des composants du lait sur la biodisponibilité de la thiamine ne provenant pas du lait chez le rat. Le rôle joué par les composants du lait sur la biodisponibilité de la thiamine (ne provenant pas du lait) a été testé sur de jeunes rats carencés en cette vitamine. Un lait cru de vache (à 3,5% de matière grasse) dialysé 28 h était utilisé comme source de composants du lait. Les régimes suivants ont été testés pendant 21 j, avec au total comme source de thiamine, (A) le lait dialysé seul (équivalent à 59 µg de thiamine liée), (B) le lait dialysé (59 µg) + 256 µg de thiamine pure ou (C) 315 µg de thiamine pure. La teneur en thiamine hépatique, l'activité transcétolase des érythrocytes (ETK) et l'effet du pyrophosphate de thiamine (TPP) sur l'activité ETK ont été choisis comme critères d'évaluation. Le groupe B recevant le lait dialysé supplémenté en thiamine pure présentait significativement ($P < 0,05$) la plus grande teneur hépatique totale en thiamine de la meilleure récupération de l'activité ETK comparées aux autres groupes. De plus, l'effet TPP dans ce groupe B était inférieur ($19 \pm 0,34\%$) à celui des autres groupes. Les résultats obtenus, après cor-

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rection prenant en compte la vitamine liée, sur la teneur totale en thiamine hépatique et la restauration de l'activité ETK, montrent que les composants du lait accroissent par un facteur supérieur à 2, la biodisponibilité de la thiamine chez le rat.

composant du lait / thiamine / biodisponibilité

INTRODUCTION

Several recent studies have indicated the existence of some factors in milk that favour the uptake of certain vitamins. Colman *et al* (1981) and Swiatlo *et al* (1990) reported greater availability of bovine and human milk folate due to the presence of certain constituents in milk. Colman *et al* (1981) further observed the beneficial effect on folate uptake of the addition of ionic calcium to the *in vitro* system of rat intestinal mucosa. In recent studies, we have observed the greater bioavailability of thiamine from cow milk and curd powders relative to pure thiamine using rat bioassay (Singh and Deodhar, 1992, 1993). This was attributed to certain milk factors facilitating the intestinal uptake of thiamine.

The aim of this study was to determine whether such factors also influence the bioavailability of non-milk thiamine in the diet.

MATERIALS AND METHODS

Chemicals

D-Ribulose-5-phosphate, barium salt and thiamine pyrophosphate chloride (TPP) were purchased from Sigma Chemical Co, USA. All other chemicals were of reagent grade.

Treatment of milk

Thiamine-free cow's milk was used as the source of milk factors. Free thiamine in milk was removed as follows:

The raw cow's milk (3.5% fat) was dialysed against distilled water for 28 h at 4°C with frequent changes of distilled water. This treatment completely removed free thiamine, which constituted 80% of the total milk thiamine (Singh and Deodhar, 1993) from the milk. The dialysed milk left in the dialysis bag was used as the source of milk factors in the rat bioassay.

Bioassay procedure

Bioavailability of non-milk thiamine in the diet was determined using thiamine-depleted rats.

Experimental animals

Thirty-two male albino rats (Wistar strain) weighing \approx 65 g were depleted of thiamine by feeding a thiamine-free diet (table I) for 25 d. One group of 8 rats was killed at the beginning of repletion (d 0) to obtain baseline data for total hepatic thiamine content, erythrocyte transketolase (ETK) activity and TPP effect. Twenty-four rats were randomized to different repletion test diets with 8 rats per group and were housed individually in anodized aluminium cages. All rats received thiamine-free basal diet *ad libitum* over a 21-d repletion period. Beside this, each day groups A and B received an equivalent of 45 ml dialysed milk and groups B and C received 12.2 and 15 μ g respectively of pure thiamine in solution.

Tissue collection

Before repletion one group of 8 rats was killed and after repletion the remaining groups were killed. A portion of whole blood was collected by cardiac puncture using sodium citrate (3.8%) as anticoagulant. Erythrocytes were separated by centrifuging blood samples at 1 500 rpm for 15 min at 4 °C as described by Brin *et al* (1960). Washed erythrocytes were diluted with an equal volume of chilled deionized distilled water and allowed to lyse.

The liver was excised, then blotted and quickly washed in ice-cold saline and weighed. A portion of the liver was taken for thiamine analysis. The remaining portion of the liver was homogenized (10% homogenate) in chilled deionized distilled water.

Determination of thiamine content

Thiamine content in the milk and dialysed milk was determined fluorometrically according to Kirk (1974) using a photofluorometer (Coleman 12A). Hepatic thiamine content was determined fluorometrically according to Freed (1966).

Transketolase activity and TPP effect (%) determination

ETK activity and TPP effect after the addition of TPP (50 µg) to the hemolysate were determined according to Brin (1966) and Ranhotra *et al* (1985). The homogenate was diluted (1:50) with 0.015 mol/l phosphate buffer, pH 7.4. The TPP effect (%) was calculated as given below:

$$\text{TPP effect (\%)} = \frac{\text{Hexose formed after TPP addition} - \text{Hexose formed without TPP addition}}{\text{Hexose formed without TPP addition}} \times 100$$

The hexose content formed during a 60-min reaction at 38°C was determined by the anthrone method as modified by Brin *et al* (1960).

Protein content was estimated according to Lowry *et al* (1951).

Statistical analysis of the data was carried out according to Snedecor and Cochran (1980). One-way classification of analyses of variance was used for testing treatment differences.

RESULTS AND DISCUSSION

Ameliorative rather than preventive action of thiamine from test materials was taken as the measure of biological availability in thiamine-depleted rats. Thiamine was administered orally at a sub-optimal level of intake. This was hypothesized to favour maximum absorption of the vitamin (Ranhotra *et al*, 1985).

Hepatic thiamine content and ETK activity were shown to respond positively to graded but suboptimal levels of dietary thiamine in thiamine-depleted rats (Ranhotra *et al*, 1985). The relative enhancement of ETK by saturation with TPP *in vitro* has also been demonstrated to be a sensitive

Table 1. Composition of the basal (thiamine-free) diet (%).

Composition du régime de base sans thiamine (%).

Ingredient	Quantity
Starch	35.5
Sugar	35.5
Casein (vitamin-free)	20
Refined groundnut oil	4
Salt mixture ^a	4
Vitamin mixture ^b	1

^a According to the AOAC (1975); ^b thiamine-free vitamin mixture consisted (mg/100 g of diet), menadione (0.5 mg); choline (200 mg); *P*-amino benzoic acid (10 mg); inositol (10 mg); niacin (4 mg); Calcipantothenate (4 mg); riboflavin (0.8 mg); pyridoxine HCl (0.5 mg); folic acid (0.2 mg); biotin (0.4 mg); vitamin B₁₂ (0.003 mg); vitamin A (2 000 IU); vitamin D (200 IU); vitamin E (10 IU) and glucose to make 1 g of the vitamin mixture.

indicator of thiamine nutritional status of the animal (Neumann *et al*, 1979). These indicators were chosen in this study.

Data on hepatic thiamine content, ETK activity and TPP effect (%) are given in table II. Dietary thiamine intake during repletion in groups B and C was 315 µg. In group A (receiving dialysed milk alone), the intake was 59 µg, since only a small percentage of total milk thiamine was present in bound form in the dialysed milk.

The liver is the major site of thiamine storage in the body. The repletion of thiamine-depleted rats resulted in a significant increase ($P < 0.05$) in total hepatic thiamine content in all groups as compared to baseline data on d 0. However, the in-

crease was significantly ($P < 0.05$) more pronounced in group B than in group C. Similarly, the restoration of ETK activity in group B was significantly greater than in group C.

Estimate of the influence of milk factors on biochemical indicators

The influence of milk factors in the dialysed milk on the bioavailability of non-milk thiamine was further ascertained from these data on the basis of a direct relationship between total dietary thiamine up to 600 µg during repletion and hepatic thiamine content as well as ETK activity, as shown by Ranhotra *et al* (1985).

Table II. Total hepatic thiamine content, ETK activity and TPP effect (%) in thiamine deficient rats before and after 21 d repletion ^a.

Teneur en thiamine hépatique totale, activité ETK et effet TPP (%) chez des rats carencés en thiamine après 21 j de régime ^a.

Parameter	Day 0	Thiamine source in test diet			Critical difference at 5% level
		Dialysed milk (A)	Dialysed milk supplemented with 256 µg pure thiamine (B)	Pure thiamine (C)	
Total dietary thiamine intake (µg)	—	59	315	315	
Total hepatic thiamine (µg)	7.45 ± 0.50	11.19 ± 0.39 (6)	55.37 ± 1.60 (16)	31.02 ± 0.63 (8)	3.004
ETK activity, nmol hexose/mg protein/h	19 ± 0.45	27 ± 0.35 (42)	60 ± 0.18 (216)	40 ± 0.29 (110)	1.817
Erythrocyte TPP effect (%)	67 ± 1.25	51 ± 4.64	19 ± 0.34	35 ± 0.35	2.126

^a Values are averages ± SEM for 8 rats per test diet; values in parentheses are % restoration in ETK and % thiamine deposition of total dietary thiamine intake in liver after correcting for their baseline data.

^a Les valeurs sont les moyennes ± écart-type pour 8 rats par régime. Les valeurs entre parenthèses représentent le pourcentage de restauration de l'activité ETK et le pourcentage de dépôt de thiamine dans le foie par rapport à la prise totale de thiamine après correction prenant en compte la thiamine liée.

In group C repletion with 315 μg pure thiamine resulted in an increase in hepatic thiamine content by 23.57 μg above the baseline value of 7.45 μg at the beginning of repletion. Group A, repleted with dialysed milk alone (which provided 59 μg bound thiamine), showed an increase of 3.74 μg . However, repletion with 256 μg pure thiamine along with dialysed milk (group B) produced an increase of 44.18 μg after correcting for the increase due to thiamine in the dialysed milk. Thus, on repletion with identical amounts of pure thiamine with or without simultaneous dialysed milk, the presence of milk factors was found to stimulate the restoration of hepatic thiamine level by > 2-fold (229%) than that observed in its absence.

Likewise, a similar trend was observed regarding ETK activity restoration in thiamine-depleted rats. Repletion with 315 μg pure thiamine (group C) increased ETK activity by 21 units above the baseline value of 19 units at the beginning of repletion. On the other hand, repletion with dialysed milk alone (group A) increased ETK activity by 8 units. However, repletion with 256 μg pure thiamine along with the dialysed milk (group B) produced an increase of 33 units after correcting for the rise due to thiamine in the dialysed milk. It was evident that in the presence of milk factors, restoration of ETK activity was 1.9-fold greater as compared to restoration without milk factors with identical amounts of thiamine intake.

Although the beneficial effect of dialysed milk on TPP effect was apparent (table II), the derivation of meaningful estimates of the effect of milk factors was not feasible in the absence of an established correlation between dietary thiamine intake and TPP effect.

It was concluded that bioavailability of non-milk thiamine was significantly enhanced in the presence of milk factors.

Possibly, simultaneous consumption of milk along with other foods may improve the utilization of thiamine from other dietary sources. However, further studies are required to corroborate such a hypothesis.

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found (thiamine) showed an increase of 3.74 µg However, reaction with 255 µg pure thiamine along with dissolved milk (group B) produced an increase of 44.18 µg after correcting for the increase due to thiamine in the dissolved milk. Thus, on a per-portion with identical amounts of pure thiamine with or without simultaneous dissolved milk, the presence of milk factors was found to stimulate the restoration of heat-labile thiamine level by ~ 2-fold (229%) than that observed in its absence.

Likewise, a similar trend was observed regarding ETK activity restoration in thiamine-depleted rats. Reaction with 315 µg pure thiamine (group C) increased ETK activity by 21 units above the baseline value of 19 units at the beginning of reaction. On the other hand, reaction with dissolved milk alone (group A) increased ETK activity by 6 units. However, reaction with 255 µg pure thiamine along with the dissolved milk (group B) produced an increase of 23 units after correcting for the rise due to thiamine in the dissolved milk. It was evident that in the presence of milk factors, restoration of ETK activity was 1.9-fold greater as compared to reaction without milk factors with identical amounts of thiamine.

Although the beneficial effect of dissolved milk on TPP effect was apparent (table II), the definition of meaningful estimates of the effect of milk factors was not feasible in the absence of an established correlation between dietary thiamine intake and TPP effect.

It was concluded that bioavailability of non-milk thiamine was significantly enhanced in the presence of milk factors.