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Original article

Bioavailability of thiamine in cow milk and curd powders using rat bioassay

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Summary — The biological availability of thiamine (relative to pure thiamine) in cow milk and curd prepared with mixed starter cultures consisting of *Lactococcus lactis* subsp *lactis* and *cremoris* and *Propionibacterium shermanii* was assessed using thiamine-depleted young rats. The cow milk and curd samples were freeze-dried. Repletion test diets provided 15 μg thiamine/d from pasteurized cow milk, curd or pure thiamine for 21 d. Body weight gain, urinary thiamine excretion, hepatic thiamine content, erythrocyte transketolase (ETK) activities with and without added thiamine pyrophosphate (TPP) and TPP effect were determined as the response criteria for this purpose. Twenty-five-d thiamine depletion resulted in a significant ($P < 0.01$) decrease in body weight gain, decreased thiamine excretion, hepatic thiamine level, ETK activity and increased TPP effect in erythrocyte in comparison to rats receiving standard diet. Repletion with thiamine from different sources brought about a significant ($P < 0.01$) increase in body weight gain, urinary thiamine excretion, hepatic thiamine and ETK activity. TPP effect for erythrocytes declined significantly ($P < 0.01$). Restoration was more significant ($P < 0.01$) in the group receiving curd (CG) than that in the group receiving milk (MG), while it was higher in the MG group than the pure thiamine receiving group (PTG). It was concluded that biological availability of thiamine was greater from curd and milk than from pure thiamine.

bioavailability / thiamine / transketolase activity / milk / curd

Résumé — Biodisponibilité de la thiamine dans des poudres de lait de vache et de caillé évaluée à partir d'essais sur rats. *La biodisponibilité de la thiamine de lait de vache ou de caillé obtenu avec des cultures mixtes de* Lactococcus lactis *subsp* lactis *et* cremoris *et* Propionibacterium shermanii, *comparée à celle de la thiamine pure, a été testée sur de jeunes rats carencés en thiamine. Les échantillons de lait de vache et de caillé étaient lyophilisés. Les régimes testés apportaient 15 μg de thiamine/j sous forme de lait de vache pasteurisé, de caillé ou de thiamine pure, pendant 21 j. Le gain de poids, l'excrétion urinaire de thiamine, la teneur hépatique en thiamine, les activités transcétolase des érythrocytes (ETK) avec et sans addition de pyrophosphate de thiamine (TPP) et l'effet TPP ont été choisis comme critères d'évaluation. Une carence en thiamine de 25 j provoquait une baisse significative ($P < 0,01$) du gain de poids, diminuait l'excrétion urinaire de thiamine, le niveau de thiamine hépatique et les activités ETK, et augmentait l'effet TPP dans les érythrocytes par rapport aux rats recevant le régime témoin. La réintroduction de thiamine, quelle qu'en soit la provenance, entraînait un accroissement significatif ($P < 0,01$) du gain de poids, de l'excrétion urinaire de thiamine, de la teneur hépatique en thiamine et des activités ETK. Par contre, l'effet TPP*

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dans les érythrocytes, diminuait significativement (P < 0,01). L'efficacité de l'apport était plus significative (P < 0,01) dans le groupe recevant le caillé que dans celui recevant le lait, et également plus importante dans le groupe recevant le lait que dans celui recevant la thiamine pure. Il peut en être conclu que la biodisponibilité de la thiamine du caillé et du lait est supérieure à celle de la thiamine pure.

biodisponibilité / thiamine / activité transcetolase / lait / caillé

INTRODUCTION

Milk and several dairy products are characterized as fair sources of thiamine on the basis of their thiamine content. Such an assessment is chiefly based on the chemical analysis of this vitamin (Robinson, 1967; Lofgren and Speckmann, 1979; Deodhar, 1985). This, however, does not take into account the influence of physiological and nutritional factors on thiamine utilization in the body. A sizeable percentage of milk thiamine occurs in the phosphorylated form (Houston *et al,* 1940; Chanda, 1953). Further, over 50% of milk thiamine is bound to protein (Halliday and Deuel, 1941). At physiological dosage, fod thiamine is absorbed by active transport mechanism after its liberation from the protein complex.

Recently, Ranhotra *et al* (1985) observed decreased bioavailability of thiamine in wheat flour bread to be associated with dietary fibre and phytic acid. Such inhibitors do not occur in milk and its products. Relatively little has been reported so far on the availability of milk vitamins. Recently, we have reported better availability of vitamin A in milk and heat-concentrated khoa relative to pure vitamin A using rat bioassay. This was ascribed to the association of milk vitamin with milk fat globules (Sapre and Deodhar, 1989). Recent studies have demonstrated important nutritional significance of milk folate binding protein for bioavailability of folate *in vivo* (Tani and Iwai, 1984; Swiatlo *et al,* 1990).

This study was aimed at assessing the availability of thiamine in cow milk and curd, a fermented product, relative to pure thiamine by rat bioassay.

MATERIALS AND METHODS

Pooled samples of cow milk were obtained from the NDRI cattle yard and pasteurized by holding method (63.3 °C for 30 min). The curd was prepared according to the procedure of Rangappa and Achaya (1974) using *L lactis* ssp *lactis* and *cremoris*, and *P shermanii* as starter cultures in ratio of 1:1:1. Milk and curd samples were freeze-dried by lyophilizer (Virtis 10-MR-IR). Solids were stored in the deep-freezer at –20 °C until used.

Chemicals

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

Determination of thiamine

Thiamine content in the milk and curd samples were determined fluorometrically according to Kirk (1974) using a photofluorometer (Model Coleman 12A).

Thiamine content in the liver and urine samples were also determined fluorometrically according to Freed (1966). Urine samples were passed through an activated sand column to remove interfering compounds as described by Ramasastri (1976).

Bioassay procedure

Bioavailability of thiamine in the test material was determined according to Ranhotra *et al* (1985). Thiamine-free basal diet was formulated (table I).

Experimental animals

Thirty-two newly weaned male albino rats (Wistar strain) weighing ≈ 40 g were depleted of thiamine by feeding thiamine-free diet for 25 d. One group of 8 rats was killed at the beginning of repletion (0 d) to obtain baseline data for hepatic thiamine content, ETK activity and TPP effect. Twenty-four rats were randomized to different repletion test diets with 8 rats per group housed individually in anodized aluminium cages. Rats received thiamine-free basal diet during repletion. Animals in the milk group (MG) and curd group (CG) received 15 µg thiamine from 1.5 g milk/curd solids. In the case of the pure thiamine group (PTG), 15 µg thiamine in solution as thiamine hydrochloride was given with 2 g sucrose to ensure identical calorie intake in all groups. Food intake and body weigh were recorded for next 21 d. The weight gain was one of the response criteria to determine available thiamine values from test diets. At the end of each week during repletion, 12-h urine samples were collected by keeping animals in metabolic cages.

Table I. 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 AOAC (1975). [b] Thiamine-free vitamin mixture consisting of (mg/100 g of diet), menadione (0.5 mg); choline (200 mg); P-amino benzoic acid (10 mg); inositol (10 mg); nacin (4 mg); Ca-pantothenate (4 mg); riboflavin (0.8 mg); pyridoxine HCl (0.5 mg); folic acid (0.2 mg); biotin (0.4 mg); vitamin B_{12} (0.003 mg); vitamin A (2000 IU); vitamin D (200 IU); vitamin E (10 IU) and glucose to make up 1 g of the vitamin mixture.

Tissue collection

Before repletion 8 rats were killed; after repletion, the remaining groups were killed. A portion of whole blood was collected by cardiac puncture using sodium citrate (3.8%) as the 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 equal volume of chilled double distilled water and allowed to lyse. The liver was excised, adhering blood blotted off, quickly washed in ice-cold saline and weighed. A portion of liver was taken for thiamine analysis. The remaining portion of liver was homogenized (10% homogenate) in chilled deionized glass-redistilled water.

Transketolase activity and TPP effect determination

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

$$\text{TPP effect (\%)} = \frac{\text{Hexose formed with added TPP} - \text{Hexose formed without added TPP}}{\text{Hexose formed without adding TPP}} \times 100$$

Protein was determined according to Lowry *et al* (1951). Hexose formed during a 60-min reaction at 38 °C was estimated by the anthrone method as modified by Brin *et al* (1960).

Data were subjected to statistical analysis of variance for testing the treatment differences according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Ameliorating rather than preventive action of thiamine from test materials was taken as the measure of biological availability in thiamine-depleted rats. Thiamine in milk and curd exists in the free and in phosphorylated forms depending on milk phosphatase activity, whereas pure thiamine was administered as thiamine–HCl. Thiamine was orally administered at the dosage of 15 μg/d through test materials in this study. This level of intake was sub-optimal, and was hypothesized to favour maximum absorption of thiamine (Ranhotra *et al,* 1985).

All rats maintained on thiamine deficient diet for 25 d showed thiamine deficiency symptoms characterized by decreased food intake and a decline in body weight after an initial rise. The urinary thiamine excretion in depleted rats was reduced to traces in comparison with 3.52 ± 0.42 μg/12 h in rats maintained on stock diet. The hepatic thiamine content at this stage was 2.07 ± 0.16 μg/g liver in comparison to 10.12 ± 0.40 μg/g liver for rats maintained on the standard diet.

Thiamine repletion

Thiamine-depleted rats were fed on test diets for 21 d. This limited period was chosen to restrain complete repletion of the animals. This would enable an accurate assessment to be made on the basis of different biochemical criteria as suggested by Ranhotra *et al* (1985). Erythrocyte transketolase (ETK) activities and liver thiamine content were shown to respond positively to graded but sub-optimal levels of dietary thiamine in thiamine-depleted rats by Ranhotra *et al* (1985). The relative enhancement of ETK by saturation with thiamine pyrophosphate (TPP) *in vitro* has been demonstrated to be a sensitive measure for the detection and evaluation of thiamine deficiency in human subjects by several workers (Brin, 1966; Neumann *et al,* 1979; Duffy *et al,* 1981). Neumann *et al* (1979) showed that TPP effect < 15% indicates normal thiamine nutritional status whereas values > 25% signify thiamine deficiency. Ranhotra *et al* (1985) observed on this basis that normal thiamine nutritional status was achieved with diets containing 0.2 mg thiamine. TPP effect thus appears to be a dependable indicator of thiamine bioavailability with dietary thiamine levels < 0.2 mg. The parameters taken into account in this study, therefore, included body weight gain, hepatic thiamine level, ETK activity and TPP effect.

Growth

Data on body weight gain (table II) showed significantly ($P < 0.01$) higher body weight gain in CG rats in comparison to PTG or MG rats. The body weight gain was higher ($P < 0.01$) in MG rats than in PTG rats. The relative (relative to other groups) bioavailability of thiamine from curd was greater.

Hepatic thiamine level

Repletion of thiamine-depleted rats with thiamine from different sources resulted in a significant increase in hepatic thiamine content in all groups. This increase was, however, more pronounced and significantly ($P < 0.01$) higher in the CG rats in comparison to MG and PTG rats (table II).

Urinary thiamine excretion

Urinary thiamine excretion was negligible in thiamine-depleted rats. On repletion, uri-

Table II. Rats' response to 21-d repletion test diets *.
*Influence des régimes testés à 21 j **.

Parameters	*0 d*	*Pure thiamine*	*Milk*	*Curd*	*SEM*
Dietary thiamine/day (µg)	–	15	15	15	
Body weight gain (g)		56.13[b]	67.37[c]	79.38[d]	± 4.18
Urinary thiamine (µg/12 h)					
1st week end		0.59	0.47	0.39	± 0.11
2nd week end		0.71	1.02	1.10	± 0.15
3rd week end		1.38	1.48	1.81	± 0.19
		(18)	(20)	(24)	
Total hepatic thiamine (µg)	9.00[a]	39.20[b]	48.09[c]	61.37[d]	± 1.94
ETK activity **	19.86[a]	38.86[b]	52.22[c]	58.22[d]	± 0.56
Erythrocyte TPP effect (%)	65.87[a]	37.25[b]	25.75[c]	16.87[d]	± 2.42

* Values in horizontal rows with dissimilar superscripts are significantly different at $P < 0.01$; values are averages for 8 rats.
** Expressed in nmol hexose/mg protein/h.
Values in the parentheses denote percent of thiamine excreted.
* *Sur une même ligne, les différences entre les valeurs affectées de lettres dissemblables sont significatives à $P < 0,01$; les valeurs sont les moyennes pour 8 rats.*
** *Exprimé en nmol d'hexose/mg de protéines/h.*
Les valeurs entre parenthèses expriment le pourcentage de thiamine excrétée.

nary samples collected for the 12-h period at the end of the each week of supplementation showed increased excretion of thiamine as repletion advanced (table II). Thiamine excretion was higher (24%) in CG rats than in MG rats (20%) and PTG rats (18%) after 21 d repletion.

ETK activity and TPP effect

Data on ETK activity and stimulatory effect of TPP on ETK activity in different groups are presented in table II. ETK activity increased significantly ($P < 0.01$) in all 3 groups in comparison to the enzyme activity in thiamine-depleted rats. The activity was highest in the curd receiving group followed by milk receiving and pure thiamine receiving groups. The TPP effect was 65.8% in thiamine-depleted rats. The TPP effect decreased significantly ($P < 0.01$) in all 3 groups receiving thiamine from different sources. The percent TPP effect was 16.87 in CG rats as compared to 25.75 in MG rats and 37.25 in PTG rats (table II). Thus, at the end of 21 d repletion, TPP stimulation was lowest in the CG rats and highest in the PTG rats while the value for the MG rats laid between the 2. This suggested a greater biological availability of thiamine from curd than from milk and pure thiamine.

Data on all biochemical indicators indicate that the relative biological availability of thiamine was greater in milk as well as curd relative to pure thiamine. Further, thiamine availability was significantly greater in curd than milk. The extent of this difference could not be quantified in this study

as repletion was conducted only at a single level and warrants further study.

The present data further indicated that thiamine from milk and curd was better utilized than pure thiamine at the sub-optimal oral dosage. The absence of natural inhibitors of thiamine uptake in milk in contrast to wheat as reported by Ranhotra *et al* (1985) underlined the significance of milk and its products as sources of dietary thiamine.

The superiority of milk and curd in this regard could be attributed to some indigenous factors facilitating thiamine transport. Recenty, Colman *et al* (1981) and Swiatlo *et al* (1990) reported greater availability of bovine and human milk folate due to the presence of constituents in milk. Colman *et al* (1981) further observed a beneficial effect of added ionic calcium on folate uptake in the *in vitro* system of the rat intestinal mucosa. A thiamine-less mutant of *E coli* was reported to synthesize protein which was associated with thiamine uptake (Nishimuni and Hayashi, 1971). Greater bioavailability of thiamine observed in this study could be attributed to the presence of certain factors such as calcium present in milk and curd. Milk calcium is present in colloidal form as calcium phosphate, as calcium caseinate and its ionic form, which is better utilized than several other calcium sources. Further study regarding the above is still required.

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