Thiamine uptake in human intestinal biopsy specimens, including observations from a patient with acute thiamine deficiency

Umberto Laforenza, Cesare Patrini, Costanza Alvisi, Alide Faelli, Anna Licandro, and Gianguido Rindi

ABSTRACT Mucosal biopsy specimens obtained by routine endoscopy from 108 human subjects, including one patient with thiamine deficiency, were incubated at 37 °C in oxygenated calcium-free Krebs-Ringer solution (pH 7.5) containing tritiated thiamine and 14C-dextran as a marker of adherent mucosal water. The amount of labeled thiamine taken up was measured radiometrically. In subjects with no clinical evidence of thiamine deficiency, 1) thiamine uptake by duodenal mucosa had a hyperbolic time course, reaching equilibrium at 10 min; 2) thiamine concentrations < 2.5 μmol/L were taken up predominantly by a saturable mechanism displaying Michaelis-Menten kinetics (Km, 4.4 μmol/L and Jmax, 2.3 pmol·mg wet tissue^-1·min^-1), whereas higher concentrations were taken up by passive diffusion; 3) thiamine transport had different capacities along the gastrointestinal tract (duodenum >> colon > stomach); and 4) thiamine uptake was competitively inhibited in the duodenum by thiamine analogs, albeit with a different order of potency compared with rats, and was blocked by 2,4-dinitrophenol. In the thiamine-deficient patient, the duodenal saturable uptake was increased, with higher Km and Jmax values. In conclusion, physiologic concentrations of thiamine were transported in human small intestine by a specific mechanism dependent on cellular metabolism, whose transporters appear to be down-regulated. Am J Clin Nutr 1997;66:320-6.

KEY WORDS Thiamine, thiamine uptake, human intestinal biopsy, human thiamine deficiency, kinetics

INTRODUCTION

After a normal meal, thiamine is probably present in the intestinal lumen in free form because its phosphoesters (mainly thiamine pyrophosphate, the coenzymatic form present in tissues) can be completely hydrolyzed by different phosphatases in the gastrointestinal tract. The intraluminal thiamine concentration in humans has been estimated to be < 2 μmol/L (1). In rats, the thiamine content in the lumen of the proximal small intestine has been measured and found to be < 2 μmol/L (2). Thus, physiologic intraluminal concentrations of thiamine appear to be very low and may indicate that active transport occurs at such concentrations (1, 2).

In all animal species, including humans, the pattern of in vivo intestinal absorption of low thiamine concentrations suggests saturable transepithelial transport (3). In humans, single oral doses of thiamine > 2.5–5 mg are largely unabsorbed (4, 5) and the intestinal uptake in vivo follows Michaelis-Menten kinetics (6). The cellular mechanism of thiamine intestinal transport at low physiologic concentrations has been studied mainly in vitro in rats with use of different preparations. The entry of thiamine into the enterocyte, as evaluated in brush border membrane vesicles (7), has the following features: 1) it is not influenced by the presence of Na^+, even though in rat everted jejunal sacs thiamine transport is an Na^+-dependent process (1, 8); 2) at concentrations < 1.25 μmol/L, it involves mainly a saturable mechanism whereas at higher concentrations a nonsaturable process prevails; and 3) it is competitively inhibited by different thiamine derivatives and analogs. On the other hand, the exit of thiamine from the enterocyte, as evaluated in basolateral membrane vesicles (9), is Na^+-dependent, being directly coupled to ATP hydrolysis by Na^+-K^+-ATPase and hence to the functional integrity of this enzyme, and is also significantly inhibited by different thiamine structural analogs.

There have been only a few studies on thiamine uptake in vitro by the human intestine, an experimental model that could allow a better analysis of transport mechanisms and a useful approach to its practical evaluation in humans. Using surgical specimens and labeled thiamine, Rindi and Ferrari (10) found that surgical specimens of human small intestine can accumulate thiamine in vitro by an active uphill mechanism, which involves phosphorylation of the vitamin. In these experiments, the transport mechanism appeared to be strictly associated with the absorbing surface. Hoyumpa et al (11), using human mucosal tissue obtained by multiple biopsy, reported that thiamine uptake involves a dual system, which is saturable at low thiamine concentrations and linear at higher concentrations. To

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our knowledge, the biopsy model has never been applied to the evaluation of thiamine intestinal transport in human pathology.

In the present study, biopsy specimens of gastrointestinal mucosa were used 1) to characterize in greater detail the mechanism of thiamine uptake in humans by determining its time course, its kinetic constants, its topographic distribution along the gastrointestinal tract, and the effect of structural analogs and different metabolic inhibitors; and 2) to investigate thiamine absorption in one case of human acute thiamine deficiency, as documented by assessment of red cell content of thiamine, its phosphoesters, and thiamine pyrophosphokinase (EC 2.7.6.2) activity.

SUBJECTS AND METHODS

The procedures followed were in accord with the Helsinki Declaration of 1975 as revised in 1983.

Biopsies

Mucosal specimens were obtained from 107 patients who had no clinical evidence of thiamine deficiency and no malabsorption symptoms, and underwent routine upper gastrointestinal endoscopy or colonoscopy for diagnostic purposes. The biopsy specimens were from the following sites: 8 from the stomach (83% healthy and 17% dyspeptic syndrome), 95 from the duodenum (37% healthy, 39% dyspeptic syndrome, 21% gastric ulcers, 2% cancer, and 1% polyposis), and 4 from the colon (50% healthy, 25% polyposis, and 25% diverticulosis). Subjects included 59 males and 48 females with a mean age of 52 yr. Mucosal samples were also obtained from one acutely thiamine-deficient patient (see below). After informed consent, one to two mucosal specimens weighing ($\bar{x}$ ± SEM) 6.9 ± 0.2 mg (range: 1.9–14.5 mg) were collected from each patient by using conventional endoscopy probes. Specimens were obtained only from mucosal areas that appeared endoscopically normal, and did not contain muscularis mucosae, as assessed (in separate samples) by using conventional histologic procedures. All samples were immediately placed in ice-cold buffered saline (150 mmol NaCl/L and 1 mmol Tris-HEPES/L, pH 7.5) until their use within ± 2 h after collection.

Thiamine-deficient patient

A 13-yl old thiamine-deficient boy was admitted to a hospital in October 1989 because of dyspnea, tachycardia, and excessive perspiration. His family history was unremarkable. Echocardiography showed septal akinesia and slight dilatation of the left ventricle. Acute myocarditis was diagnosed but its etiology remained unknown. On the next day, the onset of visual disturbances and vomiting prompted a neurologic examination, which showed nystagmus, anisocoria, and dysphonia. The patient, however, was alert and well oriented. Results from computerized tomography of his brain and examination of his cerebrospinal fluid were normal. Over the subsequent few days the cardiac symptoms disappeared and the nystagmus and dysphonia became less prominent. However, the child developed a slight left hemiparesis and had lid piosis, dysmetria, ataxia, a slight deficit of the right sixth cranial nerve, and a slight paresis of the ocular superior recti bilaterally. Chest X-rays, echocardiogram, electroencephalogram, Doppler ultrasonography of the neck vessels, and auditory, visual, and somatosensory evoked potentials were all normal.

Nuclear magnetic resonance of the brain showed hypodensity in T1 and T2 over both cerebral hemispheres, the cerebral cortex, the corpus callosum, the brainstem, and the midbrain. The hypodensity resulted from scattered vascular lesions, which, however, notably regressed after 18 mo. The cerebrospinal fluid was normal except for the presence of an oligoclonal band.

The patient was treated with methylprednisolone (20 mg/d) and a polyvitaminic preparation (Benexol B-12; Prodotti Roche, Milan, Italy) supplying thiamine hydrochloride (15 mg/d). After $\approx$15 d he recovered completely and in January 1990 was discharged from the hospital. The results of a neurologic examination 4 mo later were normal. However, in July 1991 he suddenly developed fatigue, paresthesias of the left half of the tongue, and ataxia. At readmission to the hospital, neurologic and otologic examinations showed lateral nystagmus and symptoms suggestive of impaired function of vestibular structures at the medullary and pontine level. Cerebrospinal fluid, brainstem evoked potentials, and results of an electrocardiogram, electroencephalogram, and echocardiogram were normal. Measurements of thiamine compounds and thiamine pyrophosphokinase activity in blood were made in August 1991 and repeated 5 mo later when the patient was asymptomatic (first and second determinations, respectively; Table 1). Between the two sets of determinations, thiamine hydrochloride (600 mg/d) was administered orally and a complete regression of the symptomatology was observed after 1 wk of treatment.

On both occasions the 6-min uptake of thiamine, determined from mucosal biopsy specimens, was evaluated at different initial concentrations of $[^3]$H$[^7]$thiamine in the incubation medium. The patient is currently receiving 300 mg thiamine hydrochloride orally (300 mg/d) and has remained well since initiation of thiamine therapy.

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tr>
<td>Concentrations of thiamine, thiamine monophosphate (TMP), thiamine pyrophosphate (TPP), and total thiamine and thiamine pyrophosphokinase (TPK) activity in erythrocytes in one thiamine-deficient patient and five control subjects</td>
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<tr>
<th>Erythrocytes (fmol/10⁶ cells)</th>
<th>Control subject</th>
<th>Thiamine-deficient patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiamine</td>
<td>2.31 ± 0.15⁴</td>
<td>1.66</td>
</tr>
<tr>
<td>TMP</td>
<td>4.20 ± 0.44</td>
<td>2.86</td>
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<tr>
<td>TPP</td>
<td>29.90 ± 0.40</td>
<td>14.13⁴</td>
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<tr>
<td>Total thiamine</td>
<td>36.41 ± 0.22</td>
<td>18.65⁴</td>
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<tr>
<td>Plasma (nmol/L)</td>
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<tr>
<td>Thiamine</td>
<td>25.32 ± 2.41</td>
<td>16.01</td>
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<tr>
<td>TMP</td>
<td>36.67 ± 1.67</td>
<td>17.10⁴</td>
</tr>
<tr>
<td>Total thiamine</td>
<td>61.99 ± 3.78</td>
<td>33.11</td>
</tr>
<tr>
<td>Erythrocyte TPK (nmol TPP ⋅ mg protein⁻¹ ⋅ h⁻¹)</td>
<td>0.089 ± 0.005</td>
<td>0.057⁵</td>
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⁴ $\bar{x}$ ± SEM. Control subjects were matched for age and sex.  
⁵ Significantly different from control subjects (two-sided tolerance limit, see Statistics): $^t P \leq 0.01$, $^{\bar{x}} P \leq 0.05$. 

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Incubation and uptake measurements

The mucosal specimens were gently dried on a piece of filter paper, weighed on an analytic balance, and incubated in a shaking thermostatic bath at 37 °C in test tubes containing 1 mL oxygenated calcium-free Krebs-Ringer buffer (pH 7.5, composition in mmol/L: 120 NaCl, 4.8 KCl, 1.2 KH₂PO₄, 1.2 MgSO₄, and 20 Tris-HEPES), 0.25–10 μmol [³H]thiamine/L (specific radioactivity: 27.75 GBq/mmol; Moravek Biochemicals, Brea, CA), and [carboxyl-¹⁴C]dextran-carboxyl (molecular mass: 50–70 kDa; specific radioactivity: 30 MBq/mmol; DuPont NEN Research Products, Boston) as a nonadsorbable marker for the evaluation of the volume of adhering water. In each experiment the amount of [³H]dextran taken up by the specimen provided a measure, based on standard solutions, of the volume of adhering water. The calculated amount of [³H]thiamine contained in adhering water was then subtracted from the total thiamine uptake. The biopsy specimens were rapidly filtered on cellulose nitrate microfilters (pore diameter 0.65 μm; Microfiltration System, Dublin, CA) previously saturated with unlabeled thiamine by extensive washing, under continuous aspiration on the filtration support, with a fresh solution containing 300 mmol α-mannitol, 10 mmol Tris-HEPES/L, pH 7.5, and 0.5 mmol thiamine hydrochloride/L (7). After the biopsy specimens were washed on the microfilters with 10 mL cold saline, the microfilters (containing the biopsy specimens) were removed and dissolved at 50 °C for 24 h with 1 mL Lumasolve (Lumac LSC, Olen, Belgium). The specimens were then mixed with 20 mL scintillation liquid (Lipoluma: Lumac LSC) and the radioactivity was measured to determine the amount of labeled thiamine taken up.

In each experiment, blanks were prepared to evaluate the radioactivity of labeled thiamine nonspecifically adsorbed on the microfilters by filtering the same volume of radioactive solution without biopsy specimens. Blank values were subtracted from the total radioactivity retained on the filter. Radiometric measurements were carried out by using a Tri-Carb model 2000 CA liquid scintillation counter (Packard Instruments Co, Inc., Downers Grove, IL) equipped with a program for the simultaneous reading of double labeling.

Biochemical determinations

Blood samples were collected from the thiamine-deficient patient in 0.5% potassium EDTA and stored at 4 °C until delivered to the laboratory for separation of the erythrocytes (usually within 3 h). Control subjects were five healthy male subjects of the same age as the one patient.

The content of thiamine and its phosphoesters in erythrocytes was determined according to the HPLC method of Bettendorf et al (12), as described by Rindi et al (13). The same HPLC method was used for the determination of thiamine and its phosphoesters in plasma after deproteinization with 8% trichloroacetic acid and extraction with diethyl ether. Determination of thiamine pyrophosphokinase activity in erythrocyte hemolysates was carried out according to Rindi et al (13). The protein concentration was determined according to Lowry et al (14) by using bovine serum albumin as a standard.

Materials

Unlabeled thiamine hydrochloride was obtained from Prodotti Roche; pyrithiamine bromide hydrobromide and 4'-oxythiamine chloride from Sigma Chemical Co, St Louis; Amprolium (1-[4-amino-2-propyl-5-pyrimidinyl] methyl]-2-methylpyridinium chloride) from Merck, Sharp and Dohme, Pavia, Italy; furosemide from Hoechst Italia SPA, Milan, Italy; and ouabain from British Drug House Ltd. Poole, United Kingdom. All other reagents were of analytic grade and supplied by Sigma Chemical Co and British Drug House.

Statistics

The significance of differences between means was evaluated by analysis of variance (ANOVA) followed by the Newman-Keuls Q test (15) by using the computer program PRIMER, version 1 (16). When a single determination was available (for the thiamine-deficient patient), it was considered significantly different from that of the control subjects when it was outside the 95% tolerance interval found in control subjects (17).

RESULTS

General features of human intestinal thiamine uptake

Time course

The cumulative uptake of 1 μmol [³H]thiamine/L by mucosal biopsy specimens from human duodenum showed a hyperbolic time course and reached equilibrium after 10 min of incubation (Figure 1).

Kinetic constants

For the calculation of the apparent kinetic constants Kₘ and Jₘₐₓ (maximal flux), 6 min was chosen as the latest point at which uptake values had a linear time course, even though it was at the beginning of the steady state condition. The use of 6 min for incubation allowed us to obtain a reflection of the

![FIGURE 1. Time course of thiamine uptake by biopsy specimens of duodenal mucosa from subjects showing no clinical evidence of thiamine deficiency. Thiamine uptake was measured at 37 °C in the presence of an initial gradient of 120 mmol NaCl/L (outside) and 1 μmol [³H]thiamine. S ± SEM of at least four biopsies from different subjects. wt, wet tissue.](https://academic.oup.com/ajcn/article-abstract/66/2/320/4655692/1)
thiamine uptake rate as well as metabolism even though the values were not an absolute measurement of either. This was done to economize thiamine and because of the difficulties involved in obtaining biopsy specimens. When thiamine uptake at 6 min was plotted against the initial concentrations of [1H]thiamine in the incubation medium in the presence of an initial sodium chloride gradient (outside > inside), a biphasic cumulative uptake curve was observed, which was nonlinear at low concentrations (approximately < 2.5 μmol/L) and linear at higher concentrations (Figure 2). The best fit of the curve, calculated by computerized nonlinear least-squares regression (1992; GraphPad Software, San Diego), could be resolved graphically into two components: a rectilinear component (expression of a nonsaturable mechanism, which prevailed at higher concentrations) and a hyperbolic component (expression of a saturable mechanism displaying Michaelis-Menten kinetics, which prevailed at lower concentrations). The values of the apparent kinetic constants of the saturable component were as follows: \( K_m = 4.4 \, \mu\text{mol/L} \), \( J_{\text{max}} = 2.3 \, \text{pmol} \cdot \text{mg wet tissue}^{-1} \cdot \text{min}^{-1} \). The passive permeability coefficient of the nonsaturable component \( (K_a) \), calculated according to Thomsom (18) as the slope of the linear portion of the cumulative uptake curve, was 0.06 μL · mg wet tissue \(^{-1} \cdot \text{min}^{-1} \). At an initial thiamine concentration \(< 1 \, \mu\text{mol/L}, \sim 90\%\) of thiamine uptake was accounted for by the saturable component whereas at a concentration of 10 μmol/L only 72% of the uptake was accounted for by this component.

**Uptake at different sites of the gastrointestinal tract**

In experiments performed with 0.25 μmol [1H]thiamine/L, a concentration that was transported prevailingly by the saturable mechanism, the mucosa of the duodenum showed the highest rate of thiamine uptake compared with other areas of the gastrointestinal tract. The colonic and gastric thiamine transport rates were 60% and 50%, respectively, of the duodenal rate (Figure 3).

**FIGURE 2.** Relation between thiamine concentration and thiamine uptake by biopsy specimens of duodenal mucosa from subjects showing no clinical evidence of thiamine deficiency. Thiamine uptake was measured after 6 min incubation at 37 °C with different [1H]thiamine concentrations.  ± SEM of 4–12 biopsies from different subjects. wt, wet tissue.

**FIGURE 3.** Distribution of cumulative thiamine uptake along the gastrointestinal tract of subjects showing no clinical evidence of thiamine deficiency. Cumulative thiamine uptake was measured after 6 min incubation at 37 °C from mucosal biopsy specimens with 0.25 μmol [1H]thiamine/L. ± SEM of at least four biopsies from different subjects. GB, gastric body; PA, pyloric antrum; DU, duodenum; AS, ascending colon; TR, transverse colon; DE, descending colon. *Significantly different from GB, PA, AS, TR, and DE, P < 0.05; ANOVA followed by Newman-Keuls Q test. wt, wet tissue.

**Specificity**

The specificity of thiamine uptake by the duodenal mucosa was assessed by testing the effects of the thiamine structural analogs pyrithiamine, 4'-oxythiamine, and Amprolium. These compounds were added to an incubation medium containing 0.25 μmol [1H]thiamine/L. All three thiamine analogs inhibited thiamine uptake significantly (Figure 4). However, their inhibiting potencies were different, pyrithiamine being much more potent than 4'-oxythiamine and Amprolium. At 2.5 μmol/L, pyrithiamine inhibited thiamine transport by 65% whereas Amprolium and 4'-oxythiamine inhibited transport by only 40% and 35%, respectively.

**FIGURE 4.** Potency of the thiamine structural analogs 4'-oxythiamine, Amprolium, and pyrithiamine in inhibiting thiamine cumulative uptake by biopsy specimens of duodenal mucosa from subjects showing no clinical evidence of thiamine deficiency. Thiamine uptake was measured after 6 min incubation in the absence (control) and in the presence of different concentrations of analogs. ± SEM of at least four biopsies from different subjects.
Cellular mechanism

The cellular mechanism of thiamine uptake was investigated by using compounds active at different sites of cellular metabolism and ionic transport. Only 2,4-dinitrophenol, an inhibitor of oxidative metabolism, inhibited significantly, by 40%, the transport of 0.25 μmol [3H]thiamine/L as determined from duodenal biopsy specimens. All other compounds, including inhibitors of Na⁺-K⁺-ATPase (ouabain and furosemide), failed to affect thiamine uptake significantly. Removal of Na⁺ from the medium also had no effect (Figure 5).

Thiamine uptake in the thiamine-deficient patient

Body thiamine status

The results of the two sets of biochemical determinations in the thiamine-deficient patient clearly indicated a significant degree of thiamine deficiency (Table 1). In particular, thiamine concentrations during the symptomatic phase (first set of determinations) were significantly lower than those of control subjects in both erythrocytes and plasma: the total thiamine content was 48.8% lower in erythrocytes and 46.5% lower in plasma. Pyrophosphokinase activity in erythrocytes also decreased significantly (by 36%). Associated symptoms (nystagmus, ataxia, fatigue, cardiac abnormalities, impaired vestibular function, paresthesias, and dyspnea) were typical for human thiamine deficiency (19) and the rapid and complete recovery after thiamine administration strongly supports this diagnosis. At the follow-up evaluation 5 mo later (second set of determinations), the patient was still thiamine-deficient but the deficiency was less severe as indicated by a lesser deficit in total thiamine content in erythrocytes (−19.5%) and plasma (−30.1%) and by the absence of neurologic symptoms. Erythrocyte pyrophosphokinase activity remained significantly low.

Thiamine uptake

Cumulative thiamine uptake curves in duodenal biopsy specimens from the thiamine-deficient patient and relevant control subjects were determined as described above. For each curve, the saturable component was calculated by subtracting the nonsaturable component from the cumulative uptake. In the thiamine-deficient patient the nonsaturable component was insignificant and could not be evaluated reliably.

The curves calculated for the patient displayed a course similar to that of the control subjects (Figure 6) but were shifted to the left, the degree of the shift being apparently related to the severity of the thiamine deficiency (Table 1). Values of the apparent kinetic constants $K_m$ and $J_{\text{max}}$ both increased in the patient (Figure 6). A significant inverse relation was found between thiamine pyrophosphate ($r = 0.999$, $P < 0.0001$) and total thiamine content ($r = 0.999, P < 0.033$) in erythrocytes and the $J_{\text{max}}$ constant of thiamine uptake.

DISCUSSION

When tritiated thiamine with a high specific activity was used it was possible to show that biopsy specimens of human intestinal mucosa (virtually devoid of muscularis mucosa), ranging in weight from 1.9 to 14.5 mg, were able to take up thiamine at physiologic concentrations. Thiamine was accumulated in the duodenal tissue by a dual mechanism that was predominantly saturable at low, physiologic concentrations of the vitamin (≤ 2.5 μmol/L) and largely nonsaturable (diffusive) at higher concentrations (Figure 2). These results agree with previous findings obtained in humans in vivo (6) and in vitro (11), as well as in rats both in vivo (20) and in vitro (1, 3, 7, 9). The value of the apparent kinetic constant $J_{\text{max}}$ in our specimens (2.3 pmol · mg wet tissue $^-1$ · 6 min $^-1$) was very similar to that reported by Hoyumpa et al (11) in humans (2.3 pmol · mg wet tissue $^-1$ · 3 min $^-1$). Conversely, $K_m$ was greater in our study (4.4 μmol/L) than in Hoyumpa et al’s (0.43 μmol/L).

**FIGURE 5.** Effect of 2,4-dinitrophenol (2,4-DNP), ouabain, furosemide, and Na⁺ removal (substituted with mannitol) on cumulative thiamine uptake in biopsy specimens of duodenal mucosa from subjects showing no clinical evidence of thiamine deficiency. Thiamine uptake was measured after 6 min incubation at 37 °C from mucosal biopsies with 0.25 μmol [3H]thiamine/L. $\bar{x}$ ± SEM of at least four biopsies obtained from different patients. *Significantly different from control, ouabain, Na⁺ removal, and furosemide, $P < 0.05$ (ANOVA followed by Newman-Keuls Q test). wt, wet tissue.

**FIGURE 6.** Relation between saturable thiamine uptake in biopsy specimens of duodenal mucosa and thiamine concentrations in one thiamine-deficient patient and in five control subjects matched for age and sex. Each point of the control subjects’ curve represents the $\bar{x}$ ± SEM of at least four to seven biopsies from different subjects. TD 1 and TD 2, first and second set of determinations in the thiamine-deficient patient; wt, wet tissue; a, pmol · mg wt $^-1$ · 6 min $^-1$; b, μmol/L.
μmol/L; 11), possibly because of differences in experimental conditions.

The highest rate of thiamine uptake was observed in the duodenum, followed in order by the colon and the stomach (Figure 3). This finding agrees with that of Rindi and Ferrari (10) in surgical specimens of the human gastrointestinal tract. The saturable component of duodenal uptake, evaluated by using a thiamine concentration of 0.25 μmol/L, was specific for the thiamine molecular structure because all thiamine analogs tested were competitive inhibitors of thiamine uptake; the most active was pyrithiamine, which is also the most potent inhibitor of thiamine uptake in the small intestine of rats (1, 21, 22). However, unlike the findings in human specimens, 4'-oxythiamine and Amprolium did not affect thiamine transport in rats, indicating that the saturable thiamine uptake mechanism in humans has molecular specificities that are not completely superimposable to those of rats, a finding that is explained by species differences. In both humans and rats the metabolic inhibitor 2,4-dinitrophenol (1) significantly inhibits thiamine uptake (Figure 5), indicating that the saturable component of transport is dependent on cellular metabolism, possibly through thiamine phosphorylation to thiamine pyrophosphate by thiamine pyrophosphokinase. In rats, phosphorylation plays a key role in the intestinal transport of thiamine [see review by Rindi (23)], as confirmed by Ricci and Rindi (24) in isolated enterocytes.

At variance with the findings reported by Hoyumpa et al (11), removal of Na⁺ from the incubation medium did not affect thiamine uptake. This finding and the ineffectiveness of ouabain and furosemide in reducing thiamine uptake deserve comment. Na⁺ removal and Na⁺-K⁺-ATPase inhibitors are known to inhibit thiamine's exit from rat enterocytes, as assessed in basolateral membrane vesicles (9). Na⁺ removal and ouabain have also been shown to inhibit thiamine net transport across everted jejunal sacs of both frogs (8) and rats (1). However, they have little effect on thiamine content, which is mainly in a phosphorylated form, in the intestinal wall of everted jejunal sacs (8). Our biopsy mucosal preparation allowed both thiamine uptake and tissue content to be evaluated but not the controlminal exit of thiamine, which is the only Na⁺-dependent step of thiamine transport (9). This may explain the present findings.

With respect to the observations made in the thiamine-deficient patient, contents of thiamine compounds in blood were measured because they provide a more sensitive and reliable index of body thiamine status than does erythrocyte transketolase activity (25, 26). These measurements unequivocally showed that the patient suffered from acute thiamine deficiency, which was more severe at the time of the first set of measurements (Table 1) when the presence of clear-cut symptoms strongly supported the diagnosis. The observation that erythrocyte thiamine pyrophosphokinase activity was significantly lower both at initial evaluation and at follow-up, when symptoms were no longer present, agrees with previous evidence that thiamine deficiency is associated with reduced thiamine pyrophosphokinase activity both in human erythrocytes (13, 27) and in rat tissues (28, 29). The cause of the deficiency in our patient remains unknown, but an impairment of intestinal thiamine absorption was excluded as a reason. In fact, the saturable component of thiamine uptake was higher in the patient than in control subjects (Figure 6), a trend that was also present at follow-up, though in a less evident manner. The higher rate of uptake was reflected in the apparent kinetic constant Jₘₐₓ, an index of the number of active thiamine transporters, which was higher in the patient than in the control subjects (Figure 6). The Kₘ value also increased in the patient when ill (first set of determinations), indicating a reduction in the affinity of the thiamine carrier. These results show that thiamine deficiency in humans may enhance the capacity of thiamine intestinal uptake, suggesting that thiamine intestinal transporters are down-regulated by thiamine concentrations in the body as proposed for rats (30). Note that in humans the intestinal transporters of other nutrients, including iron and calcium, are known to be down-regulated (31, 32).

The proposed down-regulation, indeed, is based on the findings in a single case and further data are necessary to clarify the relation between thiamine deficiency and intestinal thiamine uptake in humans. Despite this, the present observations appear to be the first reported and it is interesting that in rats dietary thiamine deficiency also increases thiamine transport in vitro (33, 34).

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REFERENCES