The Thiamine Status of Adult Humans Depends on Carbohydrate Intake

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Abstract: Thiamine requirements for humans are generally expressed as absolute values per day (mg/d) or in relation to total caloric intake. Limited data are available on the relation between thiamine requirements and the intake of carbohydrates. This study was performed to investigate the influence of stepwise increases of carbohydrate intake on the status of thiamine in healthy volunteers under isocaloric conditions.

During an adaptation phase of four days, the carbohydrate intake of twelve healthy volunteers (6 male, 6 female) was 55% of total energy intake. During the subsequent intervention periods, carbohydrate intake was increased to 65% of total energy for four days and to 75% for another four days. Thiamine intake, total energy intake, and physical activity were kept constant throughout the study. HPLC analysis was used to measure thiamine in plasma, urine and feces. Erythrocyte transketolase activity (ETK) was determined enzymatically.

During the intervention periods thiamine decreased significantly ($p < 0.05$) in plasma (from 19.3 ± 3.3 to 16.4 ± 4.0 nmol/l) as well as in urine (from 72 ± 56 to 58 ± 21 µmol/mol creatinine). ETK and feces content of thiamine remained unchanged.

An increase of dietary carbohydrate intake from 55% to 65% and 75%, respectively, of total caloric intake for four days per period at isocaloric conditions causes a decrease of plasma and urine levels of thiamine without affecting enzyme activities.

Key words: Thiamine status, plasma-, erythrocyte-, urine-, feces level, carbohydrate-rich diet, human subject

Introduction

Thiamine (vitamin B_1) requirements for humans are generally expressed as absolute values per day (mg/d) or in relation to total caloric intake (0.12 mg thiamin/MJ or 0.5 mg/1000 kcal) [1]. As a coenzyme, thiamine pyrophosphate catalyzes the activity of transketolase in the pentose phosphate pathway, as well as the oxidative and non-oxidative decarboxylation of α-keto acids by dehydrogenase complexes [2, 3]. Since the latter enzyme is mainly involved in the metabolism of carbohydrates, thiamine requirement may be more closely related to the intake of carbohydrates than to total energy intake. Therefore, an increased proportion of carbohydrates in the diet, as recommended in several sport disciplines [4], could result in an inadequate thiamine status, especially if high amounts of nonsupplemented commercial carbohydrates are used. So far only limited data are available on this topic, since earlier studies investigated mainly thiamine requirements in relation to caloric intake [5].

This study was performed to evaluate the influence of stepwise increases of carbohydrate intake on the status of thiamine (determined by blood, urine and feces analysis) in healthy volunteers under defined isocaloric conditions and unchanged level of physical activity.

Subjects and Methods

Subjects

For this study twelve (six male, six female) healthy volunteers aged 25–30 years with a body-mass index of 22 ± 2.1 kg/m^2 and usual physical activity were investigated. Two additional participants did not complete the study because of problems adhering to the dietary protocol and were therefore not included in the analysis. None of the subjects took vitaminsupplements for at least 2 months prior to the study. Informed consent was obtained from all subjects. The study was approved by the Ethics Committee of the General Hospital of the University of Vienna (EK Nr. 094/2000).

Study Plan

The study plan included 3 successive phases of four days each. During an adaptation period (phase I) of four days, subjects were instructed to avoid the intake of thiamine- and riboflavin-rich foods and to keep a dietary protocol. Based on the intake of 55% of carbohydrates from total energy during the adaptation phase, the carbohydrate intake was increased to 65% of total energy intake during the first intervention period (phase II) lasting four days, and further increased to 75% of total energy intake for another four days (phase III). Intake of thiamine, and total energy intake, were kept constant throughout the study (Table 1). Blood, urine and feces samples were taken at four time points: before (T0) and at the end (T1) of phase I, as well as at the conclusion of phase II (T2), and phase III (T3). The following parameters were analyzed: thiamine in plasma, urine, and (except for T0) feces; transketolase activity in erythrocytes (ETK); and lactate, glucose, total lipids, total cholesterol, HDL-cholesterol, and triglycerides in plasma.

Dietary intake

Dietary intake throughout the study was assessed by weighing and recording food consumption. All participants were given clear instructions on how to record dietary intake. Lunch was prepared and consumed at the Institute of Nutritional Sciences and dinner was prepared at the Institute and was taken home by the participants. Food consumption was converted into total energy and nutrient intakes by using the EWP 3.2 ("Ernährungswissenschaftliches Programm", Dato-Denkwerkzeuge, Vienna, Austria) based on the national German/Austrian food composition data base BLS 2.1 [6].

Sample collection

After an overnight fast, blood samples were collected into heparinized tubes (and fluoride/EDTA tubes for determination of lactate and glucose). After centrifugation at room temperature for 20 min at 3000 rpm, plasma was aliquoted and stored at –20°C. The sediment (packed erythrocytes) was washed with isotonic phosphate buffer pH 7 three times, aliquoted and stored at –20°C. Urine and feces were collected for 24 hrs before blood collection. The 24-hr urine volumes were recorded and aliquots stored at –20°C. Feces samples were weighed, homogenized, and immediately analyzed. During each collection period, urine and feces were stored in dark boxes under refrigeration.

Table 1: Energy and vitamin intakes during the adaptation and intervention periods

	Adaptation period	Intervention periods	
	55% Carbohydrates	65% Carbohydrates	75% Carbohydrates
	X ± SD	X ± SD	X ± SD
Energy (MJ)	9.1 ± 3.0	10.1 ± 0.9	9.5 ± 0.9
Energy (MJ) female	7.3 ± 2.0	9.3 ± 0.6	8.8 ± 0.4
Energy (MJ) male	10.9 ± 2.9	10.8 ± 0.4	10.2 ± 0.4
Carbohydrates (%)	55 ± 8	63 ± 2	74 ± 1
Fat (%)	31 ± 7	26 ± 2	15 ± 3
Protein (%)	14 ± 2	11 ± 1	11 ± 2
Vitamin B_1 (mg/MJ)	0.13 ± 0.05	0.10 ± 0.01	0.11 ± 0.01
Vitamin B_2 (mg/MJ)	0.18 ± 0.05	0.13 ± 0.02	0.12 ± 0.02
Vitamin B_6 (mg/MJ)	0.17 ± 0.05	0.16 ± 0.04	0.20 ± 0.06
Vitamin C (mg/MJ)	14.5 ± 9.0	11.6 ± 2.3	19.2 ± 6.4
Retinol equivalents (mg/MJ)	0.17 ± 0.27	0.27 ± 0.12	0.10 ± 0.02
Retinol (mg/MJ)	0.12 ± 0.24	0.05 ± 0.01	0.03 ± 0.01
β-carotene (mg/MJ)	0.34 ± 0.25	1.34 ± 0.74	0.41 ± 0.11
Vitamin E (mg/MJ)	1.14 ± 0.30	2.10 ± 0.96	1.42 ± 0.68

Analytical methods

Reagents: Thiamine pyrophosphate (TPP) to serve as the standard was obtained from Fluka (Buchs, Switzerland). All other chemicals were obtained from Merck (Darmstadt, Germany) or Ridel-de Haen (Seelze, Germany).

High-performance liquid chromatography

Thiamine was determined in plasma, urine and feces extracts according to the slightly modified HPLC thiochrome method with post-column derivatization by Kimura and Itokawa [7]. For extract preparations of feces, samples were autoclaved with H_2SO_4 for 30 minutes at 120°C. Intra-assay variations for thiamine in plasma, urine and feces were 4%, with inter-assay variation ranging between 5% and 7%. The detection limit, defined as the lowest quantitatively measurable concentration, was 0.25 ng/ml (0.67 nmol/l). Erythrocyte transketolase activity (ETK) measurement, based on the thiamine diphosphate stimulation effect, was determined according to the method of Bitsch [8]. Normal values were defined as follows: transketolase activation > 1.25 deficient, 1.15–1.25 borderline, < 1.15 adequate [5].

Total cholesterol, HDL-cholesterol, and triglycerides in plasma were determined enzymatically by using commercial assays (Cholesterin/CHOD-PAP-method and Triacylglycerol/GPO-PAP-method) and *total lipids* were determined photometrically using the commercial Merckotest 3321. LDL-cholesterol was calculated according to the formula by Friedewald (LDL-cholesterol= total cholesterol – HDL-cholesterol – triglycerides/5). *Plasma glucose and lactate* were measured photometrically by use of the glucose/GOD-Perid method (Roche Diagnostics, 124 028) and an enzymatic assay (Boehringer Mannheim, 256 773), respectively. *Creatinine* was measured in fresh urine using a commercial test (Jaffe method with deproteinization).

Statistical analysis

SPSS for Windows (version 10.0) was used for all statistical procedures. The Friedman test was performed to test for differences between the three time points of the intervention period: beginning (T1) and end (T2) of phase II (65% intake of carbohydrates) and end (T3) of phase III (75% intake of carbohydrates). Differences were regarded as statistically significant at $p < 0.05$. The Mann-Whitney U-test was used to test differences between genders at each time point (T0-T3) and considered statistically different at $p < 0.01$ in order to control for multiple testing.

Results

Thiamine intake was unchanged throughout the study, as can be seen in Table 1. None of the investigated thiamine parameters showed significant gender differences.

At the start of the study (T0), plasma concentrations of thiamine ranged from 17.5 to 35.9 nmol/l, with a mean value of 21.3 ± 4.9 nmol/l (Fig. 1). After the four-day adaptation period (T1), mean thiamine concentrations were slightly lower (19.3 ± 3.3 nmol/l), but were decreased

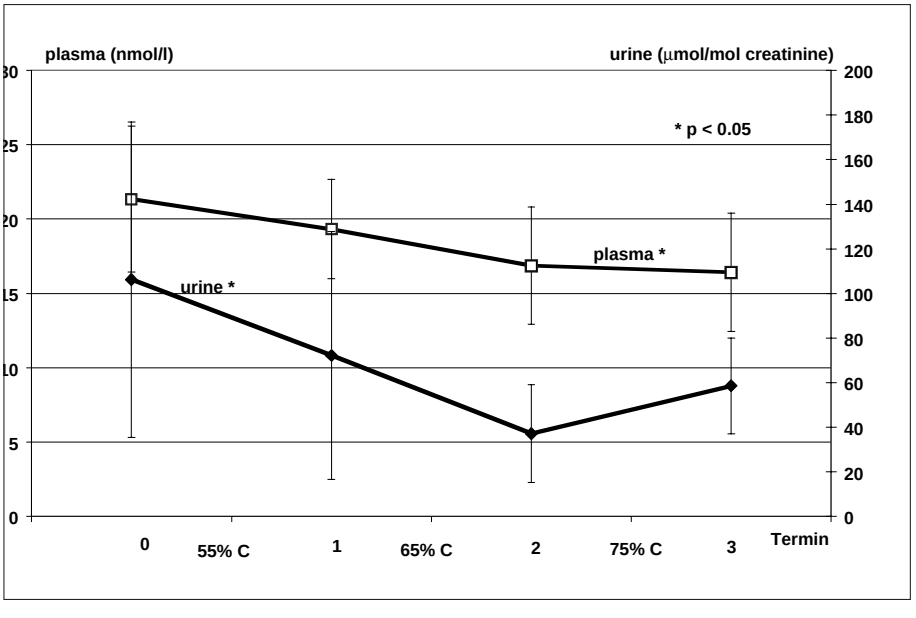

Figure 1: Thiamine concentrations in plasma and urine.

significantly ($p < 0.05$) during the intervention period to 16.4 ± 4.0 nmol/l (T3).

The mean transketolase activation at the start of the study was 1.12 ± 0.10, with a range from 1.00 to 1.36 (Table 2). No changes were observed during the adaptation phase (I) and no significant differences were found after increasing carbohydrate intake to 65% (T2) and 75% (T3) of total energy (Table 2).

The mean urinary excretion of thiamine (Fig. 2) was 106 ± 71 µmol/mol creatinine at the start of the study, and 72 ± 56 µmol/mol creatinine at the end of the adaptation period (phase I). Significant changes ($p < 0.05$) were found by increasing dietary carbohydrates. With the increased intake of carbohydrates up to 65%, the 24-hr urinary excretion of thiamine decreased to 38 ± 22 µmol/mol creatinine. The increase of carbohydrates in the diet up to 75% of total energy did not cause any further decrease of mean thiamine excretion in urine; in fact it increased up to 58 ± 21 µmol/mol creatinine, but remained below the initial value.

The excretion of thiamine in the feces ranged between 1.3 to 1.8 µmol/d without any statistically significant changes during the intervention period (data not shown).

Lipid parameters are shown in Table 2. They were within the normal range during the whole study period. No significant gender differences were seen at the start of the study. Mean total lipids decreased significantly during the intervention period ($p < 0.05$), especially in men, who also had a lower statistical mean value (374 ± 72 mg/dl) compared to women (496 ± 63 mg/dl) at the end of the study ($p = 0.016$). Total cholesterol remained constant during the whole study period, whereas HDL-cholesterol dropped significantly ($p = 0.01$) during the adaptation phase; this decrease less pronounced (14%) during the intervention period. There was a trend towards higher HDL-cholesterol levels in women, which was only significant ($p < 0.01$) at the end of phase II (65% carbohydrates). Corresponding to the decrease in HDL-cholesterol, LDL-cholesterol increased during the adaptation as well as during the intervention period. Also triglycerides changed significantly during the intervention period ($p < 0.01$), however there was not a continuous trend: compared to the end of the adaptation period, mean triglyceride levels were 33% lower at the end of phase II (65% carbohydrates) but increased again and were only 9% lower at the end of phase III (75% carbohydrates) (see Table 2).

Discussion

The results of this study indicate that thiamine status is related to the intake of carbohydrates in the diet. Increasing the amount of carbohydrates from 55% to 65% and further to 75% of total caloric intake, for four days per period at isocaloric conditions, causes a decrease of plasma and urine levels of thiamine, without affecting enzyme activities.

Baseline data of the investigated male and female volunteers regarding thiamine status were within the normal range. Mean (± SD) plasma thiamine concentrations at study entry were 21.3 ± 4.9 nmol/l, which is similar to data from Bötticher et al. [9] and Tallaksen et al. [10], who reported mean values of 17.6±6.4 nmol/l and 19.9 ± 5.5 nmol/l, respectively. Lower mean levels have been reported in earlier studies by Bettendorf et al. [11]: 14.1 ± 4.5 nmol/l and in one more recent study by Laschi-Loquerie [12]: 10.22 ± 63.2 nmol/l. Tallaksen et al. [10] reported no significant differences of mean thiamine levels in serum of males and females, in accordance with our data.

Thiamine concentrations in plasma and urine began to decrease during the adaptation phase, when a diet with de-

Table 2: Enzyme activity (ETK), glucose, lactate and lipid parameters

	T0	T1	T2	T3
Thiamin ETK	1.12 ± 0.10	1.12 ± 0.18	1.17 ± 0.15	1.12 ± 0.12
Glucose (mg/dl)	86 ± 9	86 ± 7	83 ± 5	82 ± 7
Lactate (mmol/l)	1.7 ± 0.7	1.5 ± 0.5	1.5 ± 0.5	1.4 ± 0.6
Total lipids (mg/dl)*	549 ± 98	507 ± 79	458 ± 77	435 ± 90
Total cholesterol (mg/dl)	169 ± 37	158 ± 31	162 ± 32	165 ± 44
HDL-cholesterol (mg/dl)*	84 ± 20	52 ± 12	45 ± 13	45 ± 14
LDL-cholesterol (mg/dl)**	63 ± 29	80 ± 26	99 ± 27	98 ± 34
Triglyderides (mg/dl)***	114 ± 66	132 ± 55	88 ± 32	120 ± 70

T0 Study entry
T1 After adaptation phase
T2 After intervention period I (65% carbohydrate)
T3 After intervention period II (75% carbohydrate)
ETK erythrocyte transketolase activity

Statistically significant changes during the intervention periods:
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$

fined intakes of thiamine (1.1 mg/d) was established without altering macronutrient composition (carbohydrates: 55% of total energy intake), indicating higher thiamine intakes prior to the study. The mean values of plasma and urinary thiamine decreased significantly during the intervention period. At the end of the 65% carbohydrate phase, mean urinary excretion was 38 µmol/mol creatinine; this is close to the reference value of 22 µmol/mol creatinine, representing the border value for marginal thiamine deficiency [5].

In contrast, erythrocyte transketolase activity remained unchanged throughout the study period. The missing alteration of transketolase activation may be due to the short study period.. It may be assumed that ETK would likely be affected during a longer intervention period, since erythrocyte transketolase activity is a marker for long-term status, whereas urinary thiamine excretion reflects recent dietary intake [5]. The course of thiamine excretion in our study group is comparable to that of a subgroup of male volunteers under partial thiamine restriction (500 µg/d), who showed a sharp decrease in urinary thiamine excretion within one week, followed thereafter by a small continuous increase for 3 weeks [13]. The decrease of urinary thiamine during the adaptation phase is very likely caused by a lower thiamine intake compared to the prestudy phase. Since thiamine intake as well as energy intake and physical activity were kept constant throughout the study, the further decrease of urinary thiamine after the adaptation period can be attributed primarily to the elevated intake of carbohydrates, which obviously increased thiamine requirements.

Dietary reference intakes for thiamine are expressed in absolute terms or related to total energy intake [1]. According to our data, the intake of carbohydrates may also be relevant in this aspect. This might be especially true for people consuming high amounts of carbohydrates, a dietary practice recommended in several sport disciplines [4]. The use of high amounts of non-supplemented commercial carbohydrates could result in an inadequate supply of thiamine. Further studies on this topic are therefore warranted.

As reviewed extensively in a recent study [14], an increase of dietary carbohydrates, accompanied by reduced fat intake, usually leads to reduction in plasma cholesterol and elevation of plasma triglycerides. In contrast, we found no changes in plasma total cholesterol and a small decrease in plasma triglycerides. However, it must be mentioned that the duration of the intervention phases in this study (8 days in total) was probably too short to allow conclusions regarding lipid parameters.

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