Plasma and platelet taurine are reduced in subjects with insulin-dependent diabetes mellitus: effects of taurine supplementation[1–3]

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ABSTRACT Plasma and platelet taurine concentrations were assayed in 39 patients with insulin-dependent diabetes mellitus (IDDM) and in 34 control subjects matched for age, sex, and both total and protein-derived daily energy intake. Platelet aggregation induced by arachidonic acid in vitro at baseline and after oral taurine supplementation (1.5 g/d) for 90 d was also studied. Plasma and platelet taurine concentrations ($\bar{x} \pm$ SEM) were lower in diabetic patients (65.6 ± 3.1 μmol/L, or 0.66 ± 0.07 mol/g protein) than in control subjects (93.3 ± 6.3 μmol/L, or 0.99 ± 0.16 mol/g protein, $P < 0.01$). After oral supplementation, both plasma and platelet taurine concentrations increased significantly in the diabetic patients, reaching the mean values of healthy control subjects. The effective dose ($\bar{x} \pm$ SEM) of arachidonic acid required for platelets to aggregate was significantly lower in diabetic patients than in control subjects (0.44 ± 0.07 mmol compared with 0.77 ± 0.02 mmol, $P < 0.001$, whereas after taurine supplementation it equaled the mean value for healthy control subjects (0.72 ± 0.04 mmol). In in vitro experiments, taurine reduced platelet aggregation in diabetic patients in a dose-dependent manner, whereas 10 mmol taurine/L did not modify aggregation in healthy subjects. *Am J Clin Nutr* 1995;61:1115–9.

KEY WORDS Plasma taurine, platelet taurine, platelet aggregation

Introduction

Taurine is a normal constituent of the human diet and is found in animal food sources (1, 2). It has been viewed both as a metabolic end product and as an essential nutrient for some animal species and may be involved in the development and function of many organs (1, 2). Cysteinsulfinic acid decarboxylase activity is relatively low in humans (3). As a result, taurine does not have to be conjugated to bile salts, resulting in reduced taurine excretion. The excretion of taurine in humans can also be decreased through an increase in bile salt conjugation to glycine (4). Regulation of taurine body content depends on various factors such as daily dietary intake, synthesis, utilization, and loss through the biliary excretory pathways and urine; the kidney being a major regulator of body taurine pools (5). Furthermore, taurine is an integral component of blood platelets (6) and it was shown in five healthy volunteers that taurine supplementation decreases collagen-induced thromboxane release and platelet aggregation (7). Moreover, other experimenters were unable to demonstrate the antiaggregating effect of taurine per se; instead, they found that taurine potentiates the effect of antiaggregating agents such as aspirin, papaverine, and sodium nitroprusside (8).

The above observations indicate a possible role of taurine in the modulation of platelet function. Some observations also suggest that taurine in some way affects glucose utilization and interacts with insulin receptors (9–13). Therefore, we focused our attention on plasma and platelet taurine concentrations in subjects with insulin-dependent diabetes mellitus (IDDM) and on the effect of taurine supplementation on platelet aggregation. Platelets were chosen because their function is somewhat changed in diabetes (14–16) and altered platelet function seems to play a role in the pathogenesis of micro- and macrovascular complications (17).

Subjects and methods

Patients who fulfilled all criteria for IDDM as described by the National Diabetes Data Group (18) and whose fasting baseline C-peptide values were undetectable were recruited as outpatients. The healthy control group was composed of hospital staff or blood donors who had no family history of diabetes mellitus and had a normal glucose tolerance test. Nutrient intake was assessed by means of a self-administered food-frequency questionnaire containing 277 of the most commonly eaten Italian foods. All meals consumed at home in the past week were recorded either qualitatively or quantitatively. Mean daily energy intake was then estimated by using a computer program for nutrient analysis (FOOD METER; Bayer Diagnostica, Milan, Italy) (19).

There were no significant differences in energy or protein intakes between control subjects and patients (**Table 1**), and consequently, the percentages of carbohydrate, lipid, and protein in the subjects' diets were similar. The control and diabetic

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TABLE 1
Characteristics of diabetic patients and control subjects[1]

	Diabetic patients (n = 17M, 22F)	Control subjects (n = 16M, 18F)
Age (y)	34 ± 1.8	38 ± 2.6
Duration of diabetes (y)	9.1 ± 0.18	—
Body mass index (kg/m^2)	24.1 ± 0.37	23.8 ± 0.37
Plasma glucose (mmol/L)	8.1 ± 0.1[2]	4.9 ± 0.01
HbA_{1C} (%)	6.9 ± 0.04[2]	4.4 ± 0.18
Albumin excretion rate (μg/min)	15.2 ± 0.6	13.2 ± 0.03
Daily energy intake (kJ · kg^{-1} · d^{-1})	120.6 ± 3.94	136.8 ± 6.51
Protein energy intake (kJ · kg^{-1} · d^{-1})	14.9 ± 0.54	15.5 ± 0.64

[1] $\bar{x}$ ± SEM.
[2] Significantly different from control subjects, P = 0.0001.

groups differed in plasma glucose and glycosyl hemoglobin (HbA_{1C}) (Table 1). There were no statistically significant differences in serum cholesterol, high- and low-density-lipoprotein cholesterol, triglyceride, apolipoprotein A-I and B, serum and urine uric acid, and plasma creatinine concentrations between the diabetic and control subjects (data not shown). In addition, there were no differences in white and red blood cell counts and platelet count as well as in hematocrit, hemoglobin, blood pressure, and heart rate measurements. After their electrocardiograms and medical histories were evaluated, the patients showed no evidence of cardiovascular disease. None had signs of clinical retinopathy as judged by retinal color fundus photographs of two macular- and optic disc–based fields and by fluorescein angiographs. No patients had neuropathy. Patients who had received medication other than insulin 2 wk before the start of the study were not admitted. Those with body mass index (BMI, kg/m^2) > 28, HbA_{1C} > 8%, urinary albumin excretion rate (AER) > 20 μg/min, and blood pressure > 140/90 mm Hg were rejected. Informed consent was obtained from all subjects after explanation of the study. This study was approved by the local Ethical Committee at the Spedali Riuniti of Pistoia.

Blood samples were obtained after a 12-h overnight fasting period and in diabetic patients samples were obtained before insulin administration. Samples were subjected to taurine determination, platelet aggregation, and standard hematological and serum biochemical tests. No patient experienced hypoglycemic reactions within the 24 h before blood sampling. Throughout the study, patients consumed a constant isoenergetic and isoproteic diet at home, which was monitored by questionnaire, and their weights did not change. Patients received 500 mg taurine three times a day (breakfast, lunch, and dinner) and this supplementation was continued for 90 d. Compliance checks were carried out by tablet counts on each visit. Patients were visited every month and all adverse side effects were recorded whether taurine-related or not. The study was completed by 35 diabetic patients (4 dropped out).

Blood samples were collected in plastic test tubes containing 3.8% sodium citrate (1:9 by vol). Platelet-rich plasma and platelet-poor plasma were prepared as previously described (8). Platelet aggregation was measured by using a PA-322s aggregometer (Daichii, Kyoto, Japan) in accordance with the method described by Born and Cross (20), using arachidonic acid as the aggregating agent and evaluating both maximal aggregation values and rate. In in vitro experiments performed in four samples that were randomly selected from control subjects and diabetic patients who were taurine-free, taurine was added to platelet-rich plasma 10 min before the addition of arachidonic acid. In a second series of experiments, aggregation was measured in vitro before and after taurine supplementation. Taurine determinations were carried out with platelet-rich plasma and plasma treated according to Hayes et al (7). Taurine was quantitated in duplicate by HPLC using homoserine as the internal standard after derivatization with o-phthalaldehyde (21). Retention time was estimated at 28 min. The plasma glucose concentration was determined by using the glucose oxidase method. HbA_{1C} was measured by HPLC (Bio-Rad Laboratories, Richmond, VA). Radioimmunoassay (Biodata, Rome) was used for the determination of albumin excretion in the urinary samples collected within the 24-h period. The results were expressed as the mean value of three samples of the previous 6 mo. Platelet protein content was measured by using the Coomassie protein assay technique (Pierce, Amsterdam).

Statistical analysis was performed with the SAS statistical package for personal computers (22). All data are expressed as means ± SEM. Student's t test for paired data was used to determine whether taurine concentrations of patients were significantly different before and after supplementation. Unpaired t statistics were used to compare taurine concentrations between the control and diabetic groups. The correlation of taurine concentrations with other biochemical and functional indexes was assessed by applying simple regression analysis. Variance analysis was used to analyze differences in dose-response curves. P values ≤ 0.05 were considered to be significant.

Results

Diabetic patients had low plasma and platelet taurine concentrations when compared with healthy subjects matched according to age, sex, and body mass index, and energy and protein intakes (**Table 2**). Taurine supplementation restored platelet and plasma taurine concentrations in the patients (Table 2).

Platelets recovered from diabetic patients required less arachidonic acid to aggregate [effective $dose_{50}$; (ED_{50}) = 0.44 ± 0.07 mmol] compared with those from healthy subjects (ED_{50} = 0.77 ± 0.02 mmol; $P < 0.001$). In vitro experiments performed on platelet-rich plasma from diabetic patients after 3 mo taurine supplementation showed that the dose-response

TABLE 2
Plasma and platelet taurine concentrations in control subjects and in diabetic patients who completed the study, at baseline and after taurine supplementation (1.5 g for 90 d)[1]

	Control subjects (n = 34)	Diabetic patients (n = 35)	
		At baseline	After taurine supplementation
Plasma taurine (μmol/L)	93.3 ± 6.3	65.6 ± 3.1[2]	126.8 ± 12.3[3]
Platelet taurine (mol/g protein)	0.99 ± 0.16	0.66 ± 0.07[2]	0.99 ± 0.14[3]

[1] $\bar{x}$ ± SEM.
[2,3] Significantly different from control subjects: [2] $P < 0.001$, [3] $P < 0.01$.

curve of arachidonic acid shifted to the right (**Figure 1**). The percentage of nonresponders was 26.6%. In vitro, the addition of taurine dose-dependently reduced platelet aggregation induced by 0.6 mmol arachidonic acid/L in platelet-rich plasma from diabetic patients, whereas the addition of 10 mmol taurine/L did not modify aggregation in healthy subjects (**Figure 2**).

Blood pressure, heart rate, and insulin intake remained unchanged throughout the study. Four patients dropped out: one withdrew for dietary reasons, two took aspirin-like drugs, and one stopped the treatment 10 d before the completion of the study.

An inverse correlation existed between log plasma taurine concentrations and HbA_{1C} at baseline in diabetic patients who completed the study ($y = -0.05x + 2.08$; $r = 0.60$; $P < 0.001$; $n = 35$). No correlation was found between taurine concentrations and age; sex; duration of illness; body mass index; glucose and triglyceride concentrations; total, LDL-, or HDL-cholesterol concentrations; blood pressure; daily insulin intake; or AER. The correlation between log taurine plasma concentrations and HbA_{1C} disappeared after taurine supplementation.

Discussion

According to our data, taurine values are lower in both plasma and platelets in patients with metabolically controlled IDDM. It is well known that plasma concentrations of amino acids change in diabetic patients with poor metabolic control (23). The reason for the reduction in taurine in our group of IDDM patients with good metabolic control is a matter for debate. A 30% reduction of plasma taurine in IDDM patients with good metabolic control was also indirectly suggested by Luzi et al (24). The diet factor cannot be considered to be responsible, because both control and diabetic patients were given diets that provided the same protein intake. In addition, an alteration in taurine biosynthesis seems to be an unlikely candidate because it is of minor importance in the maintenance of taurine concentrations in humans (3). On the other hand, the intestinal absorption of taurine does not seem to be impaired, because its plasma concentrations increased after supplementation. Diabetes could bring about a change in the distribution of taurine, as shown by studies carried out with the experimen-

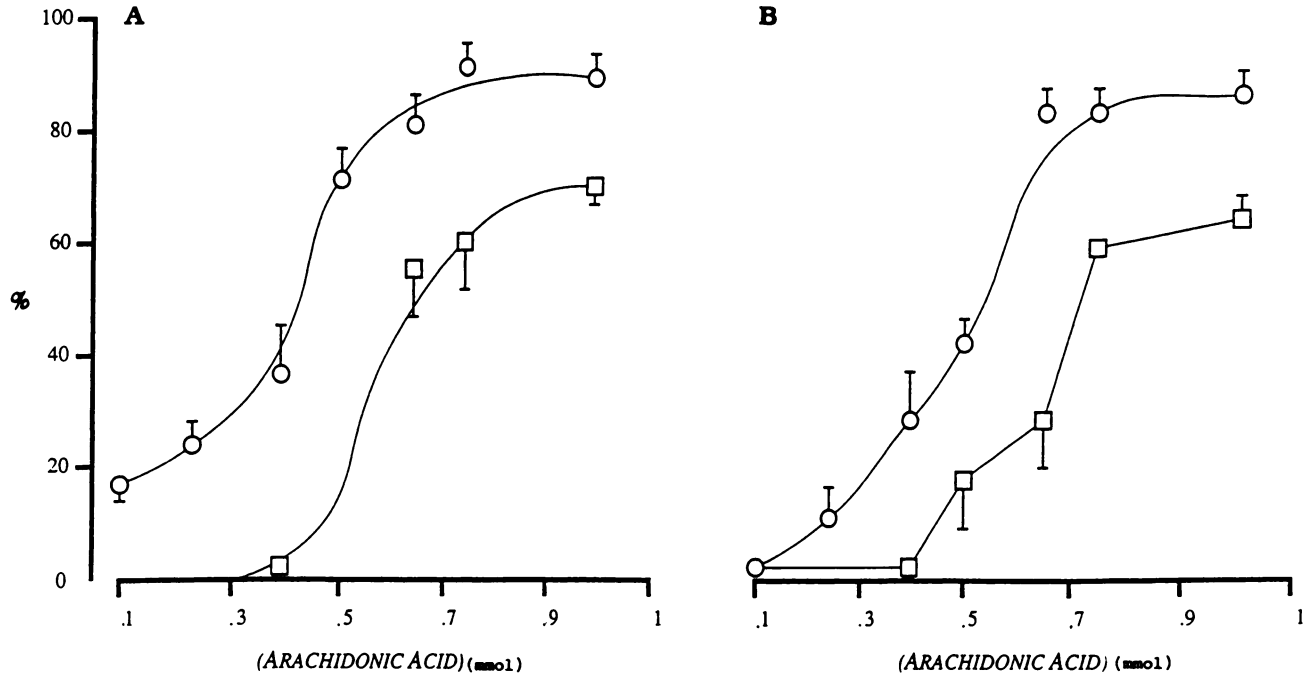

FIGURE 1. Dose-effect curve of arachidonic acid on platelet-rich plasma obtained from 35 patients with diabetes before (○) and after 1.5 g taurine/d for 90 d (□). $\bar{x}$ ± SEM. Maximal aggregation (A) and aggregation rate (B) were significantly different after supplementation, $P < 0.001$ (analysis of variance).

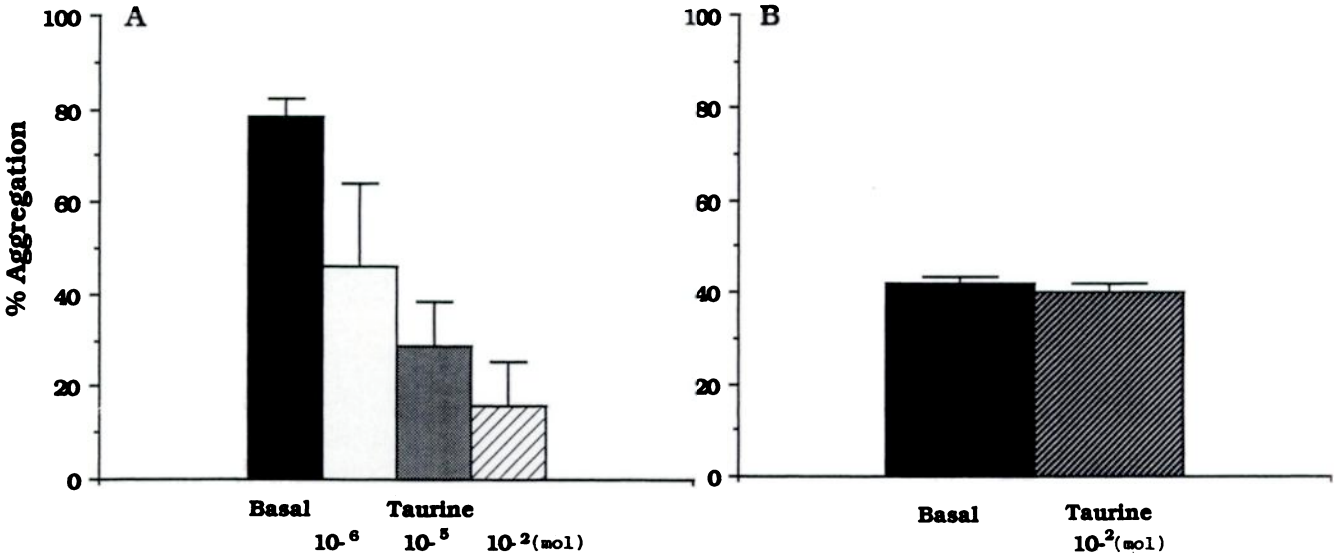

FIGURE 2. Effect of taurine preincubation on platelet aggregation induced by 0.6 mmol arachidonic acid/L in diabetic patients (A) and on platelet aggregation in control subjects (B). $\bar{x} \pm$ SEM of four independent experiments.

tal model of diabetic rats (25), despite the fact that in the present study no variations in plasma concentrations were detected. Taurine may function as a volume-regulating osmolyte, especially in human cells, where it is found in high quantities (26–28); however, compared with other osmolytes, the contribution of taurine and other amino acids to the maintenance of cell volume (at least in the human lymphocyte) is relatively small (29). Our study shows, however, that taurine decreased in both extra- and intracellular compartments, a phenomenon that cannot be linked to an osmotic effect. The absence of clinical nephropathy in our patients, as confirmed by a normal AER, excludes the hypothesis that taurine depletion may be related to renal impairment.

A higher consumption of taurine may imply an increased antihypoxic effect (30–32). In diabetes, numerous alterations decrease tissue oxygenation and perhaps an increase in taurine consumption could counteract this situation. Studies have offered compelling evidence suggesting the possible role of oxygen-free radicals in the pathogenesis of diabetic complications (33). In this context, taurine may well behave as a reducing agent because it has been shown to react with oxidants such as hypochlorous acid and to reduce lipid peroxidation (5, 34). Moreover, a protective effect of taurine administration against hyperglycemia induced by streptozotocin in mice has been described (35). This process has been pathologically ascribed to the action of oxygen-free radicals (36). It seems therefore conceivable that the reduction of taurine concentrations in IDDM is related to an increase in its consumption, because the taurine synthetic rate in humans is low (3). This unbalance between reduced synthesis and high consumption is consistent with the suggestion that taurine is a semiessential amino acid in humans (1, 2). No serious side effects were observed during taurine supplementation in the present study and these findings agree with those of Azuma et al (37) and Takahashi et al (38).

The ED_{50} values of arachidonic acid obtained in control subjects and diabetic patients before taurine supplementation are almost identical to those reported by Davi et al (15) and Halushka et al (16). Taurine supplementation significantly increases the ED_{50} of arachidonic acid and consequently reduces platelet aggregation in diabetic patients, affecting both the aggregation and the rate of aggregation. The increase in platelet aggregation in patients affected with IDDM was reversed after oral supplementation of taurine, which also happens in control subjects (7). Analogous behavior was observed in the acute replenishment of taurine in in vitro studies. The in vitro replenishment of taurine had no effect in control platelets as opposed to those obtained from diabetic patients. This indicates a difference in sensitivity to taurine between the platelets of the two groups. The in vitro action of taurine on platelets of diabetic patients is independent from the variations of glucose, lipids, and daily insulin intake, thus excluding their involvement in the antiaggregating properties of taurine. Additionally, taurine seems to be related to metabolic control in diabetic patients because plasma taurine concentration was inversely proportional to HbA_{1C}.

In conclusion, our data suggest that taurine may have direct cytoprotective effects in diabetes, although the mechanism of the taurine-induced decrease in platelet aggregation is still unknown. Its antioxidant behavior would undoubtedly impart protection to cell membranes as well as to other cellular components. Furthermore, the oral administration of taurine brings about a decrease in platelet aggregation. This may lead to a reduction in diabetic complications such as micro- and macroangiopathies, the pathogenesis of which is associated with an increase in platelet aggregation (39). In this context it is important to point out that a taurine deficiency or a decrease in taurine concentrations could lead to the emergence of retinopathy, a condition that can be brought about by two different mechanisms: a direct one through interference with retinal function (40) or an indirect one through regulation of platelet aggregation (39). Moreover, a protective effect of taurine has been described in puromycin-induced renal damage (41). This suggests a selective cytoprotective effect of taurine at the renal level. Interestingly, a deficiency in taurine in cats and foxes has been found to promote a cardiomyopathic condition (42, 43), raising the question of whether taurine depletion may have some importance in the pathogenesis of diabetic cardiomyopathy. Our findings suggest that normal concentrations of tau-

rine could be of some importance in restoring to normal the clotting disorder and consequently in preventing the vascular complications in IDDM.

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