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Effects of oral 5-hydroxy-tryptophan on energy intake and macronutrient selection in non-insulin dependent diabetic patients

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OBJECTIVE: In obese patients, brain serotonergic stimulation *via* orally administered 5-hydroxy-tryptophan (5-HTP), the precursor of serotonin, causes decreased carbohydrate intake and weight loss. Since diabetes mellitus is associated with depressed brain serotonin, hyperphagia and carbohydrate craving, we hypothesized that in diabetic patients, orally administered 5-HTP stimulates brain serotonergic activity and thus normalizes eating behaviour. To test this hypothesis, we investigated whether in diabetic patients: 1) predicted brain serotonin concentrations are depressed as a result of decreased availability of the precursor, tryptophan; and 2) oral 5-HTP is effective in reducing energy and carbohydrate intake.
SUBJECTS AND METHODS: 25 overweight non-insulin dependent diabetic outpatients were enrolled in a double-blind, placebo-controlled study, and randomized to receive either 5-HTP (750 mg/d) or placebo for two consecutive weeks, during which no dietary restriction was prescribed. Energy intake and eating behaviour, as expressed by macronutrient selection, were evaluated using a daily diet diary. Plasma amino acid concentrations and body weight, as well as serum glucose, insulin and glycosylated haemoglobin were assessed.
RESULTS: 20 patients (nine from the 5-HTP group and 11 from the Placebo group) completed the study. Brain tryptophan availability in diabetic patients was significantly reduced when compared to a group of healthy controls. Patients receiving 5-HTP significantly decreased their daily energy intake, by reducing carbohydrate and fat intake, and reduced their body weight.
CONCLUSIONS: These data confirm the role of the serotonergic system in reducing energy intake, by predominantly inhibiting carbohydrate intake, and suggest that 5-HTP may be safely utilized to improve the compliance to dietary prescriptions in non-insulin dependent diabetes mellitus.

Keywords: eating behaviour; diabetes mellitus; carbohydrate craving; tryptophan; serotonin; brain

Introduction

Non-insulin dependent diabetes mellitus (NIDDM) is a complex metabolic disorder, impingeing highly on the health and quality of life of patients, as well as greatly contributing to the costs of the national health care system worldwide.[1] It is estimated that in the US alone, more than 10 million people are diabetic,[2] and that approximately $100 billion are spent each year for the care of diabetic patients.[3] Thus, the possible therapeutic and economic benefits deriving from an improved metabolic control of diabetes become self-evident.

Both in animals and humans, NIDDM is characterized by hyperphagia[4,5] and hyperglycaemia. In the clinical setting, the control of hyperphagia is one of the mainstays of NIDDM therapy. In particular, carbohydrate craving, which is characteristically observed in diabetic patients, deserves special consideration by both patients and physicians. Unfortunately, compliance with a well-balanced diet is often an extremely difficult task to accomplish, despite the fact that patients are usually aware that this will result in a delay in both the need for exogenous insulin and the onset of major complications.

During the last two decades, evidence has accumulated suggesting that brain serotonin has an inhibitory influence on eating behaviour both in animals and humans.[6] Reported studies in favour of a role played by the serotonergic system in the pathogenesis of anorexia accompanying different diseases, further support this thesis.[7–10] Although the pathogenic mechanism(s) responsible for disturbed eating behaviour and impaired carbohydrate metabolism in NIDDM need(s) to be more precisely defined, consistent evidence suggests that the hypothalamic sero-tonergic system plays a key role in mediating hyperphagia. Experimental studies indicate that depressed hypothalamic serotonergic activity is associated with hyperphagia and obesity,[11] and that in streptozotocin-induced diabetic rats, hypothalamic

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serotonin concentrations are reduced.[12] Further evidence shows that in obese hyperphagic patients, the oral administration of 5-hydroxy-tryptophan (5-HTP), the direct precursor of serotonin, is effective in reducing energy intake and in enhancing patients' compliance to a hypoenergetic diet.[13,14] Beside its anorectic effect, brain serotonin appears to be also involved in macronutrient selection. Although controversy still exists on the role of sero-tonin in modulating selective macronutrient intake (for review, see Ref. 15), a number of animal studies have shown that the serotonergic system may modulate carbohydrate intake (for review, see Ref. 6). These findings have recently been confirmed in obese hyperphagic patients.[13,14] In these investigations, the oral administration of 5-HTP reduced the patient's energy intake by significantly inhibiting carbohydrate intake, while protein and lipid consumption was minimally affected.

Brain serotonin synthesis depends on the availability to the brain of its amino acid precursor, tryptophan (TRP).[16] In plasma, approximately 90% of this amino acid is bound to albumin, while less than 10% is free (free TRP). Although controversial, the predictability of brain TRP concentrations seems to be more appropriate when the ratio between free TRP and the other large neutral amino acids (LNAA) is considered.[8,17–19] Since plasma branched-chain amino acids (BCAA), which compete with TRP for brain entry, have been found to be increased in NIDDM patients,[20,21] we hypothesize that brain tryptophan availability and serotonergic activity are depressed in NIDDM. This might, in turn, be responsible for the hyperphagia and disturbed eating behaviour.[11,12] Consequently, the pharmacologically-induced stimulation of the brain serotonergic system might be beneficial in reducing hyperphagia, inhibiting carbohydrate intake and eventually improving the metabolic control of diabetes. To test this hypothesis, we carried out the present double-blind, placebo controlled study, which aims at investigating whether brain tryptophan availability is reduced in patients with NIDDM, and whether in the same patients the oral administration of 5-HTP may decrease carbohydrate dietary intake.

Methods

Patients

Adult patients with NIDDM (diagnosis made $\geq$3 y earlier) with a body mass index (BMI) between 25 and 30, and referred to the outpatient Nutrition Clinic of our Department, were considered for the present study. Patients with hyperlipidaemia, chronic liver or renal failure or neoplastic disease, were not included in the study. Patients adhering to a hypoenergetic diet and/or those receiving insulin treatment were also excluded.

Twenty-five patients (14 male, 11 female) aged between 35–70 y, were found eligible for the study. Among them, patients on treatment with oral hypoglycaemic drugs were invited not to withdraw or reduce their therapy.

Study design

The study design was approved by the Ethics Committee at the University of Rome 'La Sapienza', and was in accordance with the Helsinki Declaration of 1975, as revised in 1983. The three-week study period was subdivided into a baseline observation period (one week) followed by a two-week treatment period. On day −7, all patients meeting the inclusion criteria were enrolled after giving written, informed consent, and were asked to record daily, in a diet diary, their intake for the following three weeks (see below). On day 0, overnight fasting blood samples were collected for biochemical measurements (including plasma amino acid determination) and patients were examined to evaluate eating behaviour and body weight. On the same day, patients were randomly assigned to receive either 5-HTP (250 mg three times/d, $n = 12$) or placebo ($n = 13$), composed of corn starch, mannitol and magnesium stearate; (both obtained from Sigma-Tau Industries, Pomezia, Italy). The drug, which was in the form of capsules not dissolving until pH 8.6, was taken three times per day, 30 min before each meal. During the two-week treatment period, no dietary restrictions were recommended. Subjects were then followed up every week (day 7 and day 14) to evaluate eating behaviour, body weight and blood biochemistry. Also, patients were questioned for the presence of side effects, including nausea and vomiting, using a previously validated questionnaire.[13,14] To test patients' compliance to treatment 24 h urinary excretion of 5-hydroxy-indoleacetic acid (5-HIAA) was also determined on day 0, day 7 and day 14, using the chromatographic-colorimetric method described by Udenfriend *et al*.[22]

Data obtained in the study group were then compared to those simultaneously obtained in a control group of 18 gender- and age-comparable healthy subjects. Blood samples were obtained and processed as described for diabetic patients.

Biochemistry

Baseline plasma amino acid concentrations were determined using the ion-exchange technique previously described.[23] Total TRP concentrations were determined separately using the spectrophotofluorimetric method described by Denckla and Dewey[24] and revised by Bloxam and Warren.[25] The same procedure was used to estimate free TRP. Briefly, 2 ml of plasma were centrifuged at 3000 rpm for 45′ in ultrafiltration cones having 25 000 MW cut-off (Amicon CF/25 cones; Amicon Div., W.R. Grace & Co., Danvers, MA) and the ultra-filtrate tested for free TRP.

Serum glucose was measured at the beginning and at the end of the study, by the glucose oxidase method, with a glucose analyser. At the same time points, serum insulin and glycosylated haemoglobin were measured by radioimmunoassay.

Energy intake and eating behaviour

Daily total energy intake, as well as single macronutrient selection, which may define eating behaviour, were assessed by using a diet diary throughout the study period. Food diaries, including all beverages, were compiled daily by each patient, who was instructed to carefully weigh food before meals and then reweigh any left over. All reports were validated by a next of kin's signature. To avoid reported interference due to premenstrual depression, food intake measurements were not assigned to this time of the month.[26]

Statistical analysis

All data were subjected to standard statistical analysis including mean, standard error (s.e.m.), and Student's *t*-test, for both paired and unpaired data. Inter- and intra-group modifications in eating behaviour and plasma biochemistry were compared. The chi-squared test was used to statistically evaluate the prevalence of different macronutrients contributing to the daily energy intake, as calculated from the diet diaries of the two groups of patients. The minimum probability level considered for statistical significance was $P < 0.05$. Data are presented as mean $\pm$ s.e.m.[27]

Results

Five patients did not complete the study. Three patients (one in the 5-HTP group and two in the Placebo group) were not evaluated, as they only revealed at the end of the study that they were on a hypoenergetic diet. One patient in the 5-HTP group did not comply with the study protocol, as revealed by 5-HIAA urinary excretion, which was constantly within the normal range. Another patient in the 5-HTP group dropped out because of the onset of severe nausea during the first day of treatment, leading him to spontaneous withdrawal of the drug. A total of twenty patients were therefore evaluated, 11 in the Placebo group and 9 in the 5-HTP group. As shown in Table 1, both groups were comparable for gender, age, body weight, daily total energy intake and macronutrient selection.

Table 1 Baseline characteristics of the two groups studied, placebo and 5-hydroxy-tryptophan (5-HTP) groups (mean $\pm$ s.e.m.)

	Placebo (n = 11)	*5-HTP (n = 9)*
Gender (M/F)	4/7	2/7
Age (y)	55.4 $\pm$ 3.1	58.3 $\pm$ 2.3
Age (median; y)	56	61
Age (range; y)	38–70	49–67
Body weight (kg)	84.8 $\pm$ 4.8	86.2 $\pm$ 3.5
Energy intake (kJ/d)	8256 $\pm$ 564	8088 $\pm$ 472
Carbohydrate intake (g/d)	252 $\pm$ 24	263 $\pm$ 16
Fat intake (g/d)	84 $\pm$ 6	73 $\pm$ 3
Protein intake (g/d)	77 $\pm$ 6	71 $\pm$ 4

Biochemistry

Plasma total and free TRP concentrations in the 20 NIDDM patients studied were not different from those simultaneously obtained in a group of healthy volunteers serving as controls (Table 2). In contrast, plasma LNAA levels (that is, Phe, Tyr, Met, Val, Leu, Ile), competing with TRP for blood–brain barrier transport, were significantly increased in diabetic patients compared to healthy controls (Table 2). This was due to the statistically significant rise in BCAA (that is, Val, Leu, Ile) plasma concentrations (Table 2). Consequently, the plasma ratio between free TRP and LNAA, which expresses brain tryptophan availability,[6] was significantly reduced in diabetic patients when compared to healthy controls ($P < 0.01$).

Patients' compliance to the study

As expected, 24 h 5-HIAA urinary excretion in the Placebo group did not increase during the study, being stable at approximately 2–6 mg/dl. In patients receiving 5-HTP, it rose from the baseline value of 3.8 $\pm$ 0.1 mg/dl to 311 $\pm$ 21 mg/dl during the first week of treatment and 310 $\pm$ 10 mg/dl during the second week ($P < 0.001$ *vs* baseline value).

Energy intake and eating behaviour

Analysis of the mean total energy intake, as calculated from patients' reports, showed no significant changes in the Placebo group (Figure 1). In contrast, the oral administration of 5-HTP significantly reduced mean daily energy intake, which dropped from 8088 $\pm$ 472 kJ/d to 6388 $\pm$ 580 kJ/d during the first week ($P < 0.01$), and 6328 $\pm$ 528 kJ/d during the second week ($P < 0.01$) (Figure 1).

Table 2 Plasma amino acids in diabetic patients ($n = 20$) and healthy volunteers ($n = 18$) serving as controls (*$P < 0.01$ *vs* healthy volunteers). Large neutral amino acids (LNAA) plasma levels include branched-chain amino acids (BCAA) + Phe + Tyr + Met. Plasma amino acid concentrations in diabetic patients were obtained before treatment with 5-hydroxy-tryptophan (5-HTP). Data are presented as mean $\pm$ s.e.m.

	Healthy volunteers	*Diabetic patients*
Total tryptophan (μmol/l)	57.4 $\pm$ 3.7	65.0 $\pm$ 2.6
Free tryptophan (μmol/l)	4.8 $\pm$ 0.3	5.1 $\pm$ 0.2
LNAA (μmol/l)	575 $\pm$ 22	738 $\pm$ 21*
BCAA (μmol/l)	425 $\pm$ 18	543 $\pm$ 20*
Free tryptophan/LNAA ($\times 10^{-3}$)	8.58 $\pm$ 0.53	7.01 $\pm$ 0.13*

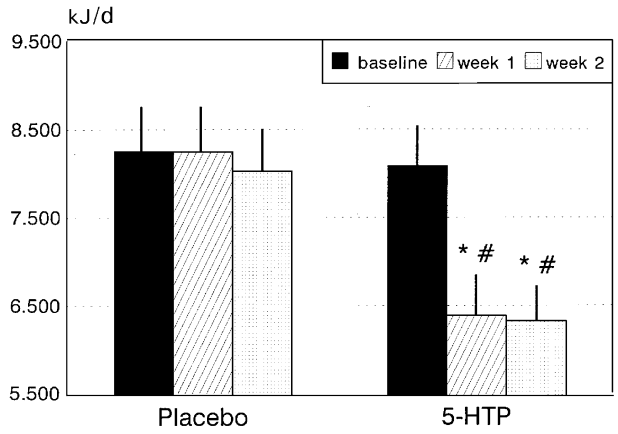

Figure 1 Mean (± s.e.m.) energy intake in the two groups studied. The oral administration of 5-hydroxy-tryptophan (5-HTP) significantly reduced energy intake when compared to the baseline period and the Placebo group (*$P < 0.01$ *vs* baseline, same group; #$P < 0.05$ *vs* Placebo, same week).

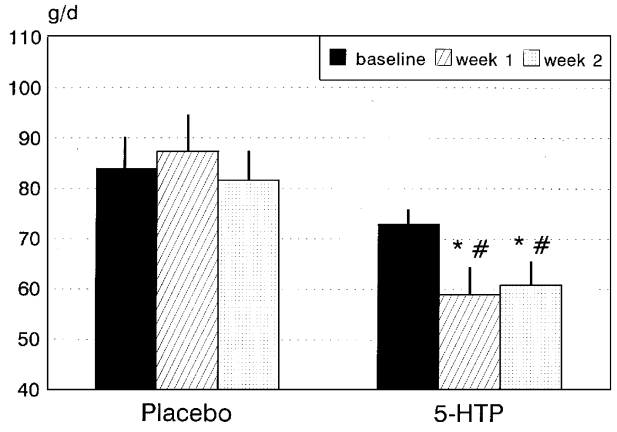

Figure 3 Mean (± s.e.m.) lipid intake in the two groups studied. The oral administration 5-hydroxy-tryptophan (5-HTP) significantly reduced lipid intake when compared to the baseline period and the Placebo group (*$P < 0.01$ *vs* baseline, same group; #$P < 0.05$ *vs* Placebo, same week).

When specific macronutrient intakes were considered, we observed that in the Placebo group, macronutrient selection did not change throughout the study period. In contrast, in the 5-HTP group, carbohydrate and fat intakes declined during the second week of treatment from 263 ± 16 g/d to 196 ± 20 g/d (Figure 2) and from 73 ± 3.2 g/d to 61 ± 4.8 g/d (Figure 3), respectively ($P < 0.01$). Thus, approximately 75% of the reduction in daily energy intake observed in the 5-HTP group, was due to a decrease in carbohydrate intake (-1100 kJ/d out of -1750 kJ/d), whereas the reduced fat intake accounted for the remaining 25% (approximately -440 kJ/d).

Body weight

Patients receiving placebo did not show any significant change in their body weight during the study period (84.8 ± 4.8 kg *vs* 84.6 ± 4.7 kg, baseline value *vs* end-of-study value, respectively). In contrast, subjects receiving 5-HTP showed a significant weight loss at the end of the two-week treatment period (86.2 ± 3.5 kg *vs* 84.1 ± 3.5 kg; $P < 0.01$).

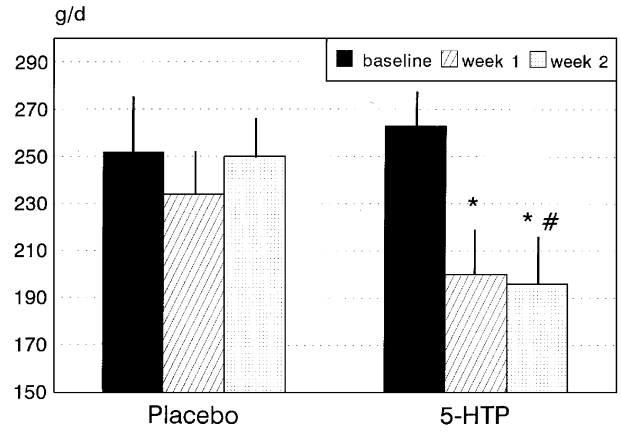

Figure 2 Mean (± s.e.m.) carbohydrate intake in the two groups studied. The oral administration of 5-hydroxy-tryptophan (5-HTP) significantly reduced carbohydrate intake when compared to the baseline period and the Placebo group (*$P < 0.01$ *vs* baseline, same group; #$P < 0.05$ *vs* Placebo, same week).

Metabolic indices

The oral administration of both placebo and 5-HTP did not modify plasma fasting glucose concentrations (Table 3). In both groups, serum insulin and glycosylated haemoglobin levels did not change significantly throughout the study (Table 3).

Discussion

The results obtained in this study show that in patients with NIDDM: 1) on the basis of the free TRP/LNAA ratio, predicted brain tryptophan availability, and thus possibly brain serotonin synthesis, is depressed; 2) the oral administration of the serotonin precursor, 5-HTP, decreases total energy intake by predominantly inhibiting carbohydrate intake, resulting in a reduction of body weight.

The association between diabetes and brain serotonin is recognized. Animal studies consistently show that in diabetic rats, brain serotonin concentrations are significantly reduced.[12,28] Moreover, long-term treatment with insulin alone or in association with tryptophan has been shown to normalize the neurochemical imbalance associated with diabetes mellitus in experimental animals.[29–31] Similar findings have been obtained in depressed diabetic patients using serotonin selective uptake inhibitors.[32,33]

Brain serotonin synthesis is directly dependent on the brain availability of its precursor amino acid TRP, which in turn appears to be influenced by the molar ratio in plasma between free TRP and the other LNAA, competing for TRP brain entry.[8,17–19] Further supporting this thesis, we have recently demonstrated in experimental animals a direct relationship between the plasma ratio free TRP/LNAA and the brain

Table 3 Serum metabolic parameters (mean ± s.e.m.) determined in both groups (placebo and 5-hydroxy-tryptophan (5-HTP)) at the beginning and at the end of the study. Glycosylated haemoglobin is expressed as % of total haemoglobin

	Placebo group		*5-HTP group*	
	Day 0	*Day 14*	*Day 0*	*Day 14*
Glucose (mmol/l)	9.3 ± 0.4	8.5 ± 0.5	8.3 ± 0.5	7.6 ± 0.5
Insulin (mU/l)	17.1 ± 2.1	20.3 ± 3	16.5 ± 3.8	12.9 ± 2.6
Glycosylated haemoglobin (HbA_{1c} %)	5.9 ± 0.6	5.8 ± 0.6	5.6 ± 0.6	4.6 ± 0.6

serotonin concentrations,[34] thus confirming previously reported data.[35] Branched-chain amino acids, which represent the major component of plasma LNAA have been found to be significantly increased in NIDDM patients,[20,21] probably secondary to insulin resistance in peripheral tissues.[36] In the present study, the significant reduction of the ratio in plasma free TRP/LNAA observed in NIDDM patients suggests that in the brain, tryptophan concentrations and thus serotonin synthesis are likely to be decreased. It is therefore conceivable that, as already observed in diabetic animals,[12] patients with NIDDM have a depressed brain serotonergic neurotransmission. Since brain
serotonin regulates energy intake and possibly macronutrient selection, we postulate that reduced tryptophan availability to the brain may contribute to the excess in energy and carbohydrate intakes of diabetic patients. This is indirectly supported by our previously reported data showing that the oral administration of 5-HTP in obese patients is effective in reducing energy intake by predominantly inhibiting carbohydrate intake, and therefore improving patients' compliance to low energy dietary prescriptions.[13,14] On the other hand, the evidence obtained in the present study that patients' eating behaviour complied well with standard nutritional advice (50% of energy intake from carbohydrates, 30% from fats and 20% from proteins) despite a reduction in predicted brain tryptophan concentrations, might reason against a selective role for brain serotonin in modulating macronutrient selection. However, it must be remembered that in the present study, patients had a history of diabetes dating at least 3 years. Thus, it is conceivable that the long-term awareness of their illness, as well as the likely frequent nutritional counselling by physicians, might have influenced their macronutrient selection, even if patients were not on a hypoenergetic diet.

Under normal conditions, depressed brain serotonergic neurotransmission causes hyperphagia.[6,11] In fact, pharmacologically-induced stimulation of the serotonergic neurotransmission in obese animals and humans results in a reduction of energy intake,[13,14,37] while enhancement of food consumption has been reported following the administration of brain serotonin receptor antagonists.[38] Moreover, in experimental animals, the brain serotonergic stimulation is usually followed by a reduction of the amount of carbohydrates in the diet.[39–43] Data obtained in the present study also validate these findings in patients with NIDDM, indicating that the pharmacological brain serotonergic stimulation decreases energy intake by predominantly inhibiting carbohydrate intake. It must be remembered, however, that as direct measurement of brain serotonergic activity was not obtained in this study, we may only infer that the effects on energy intake and macronutrient selection were mediated by an increase in serotonin synthesis within the brain, as suggested by increased urinary excretion of 5-HIAA, the final serotonin metabolite. In fact, the enhancement of serotonin synthesis in other body tissues could have contributed to the rise of 5-HIAA urinary levels. Also, we acknowledge that the impairment of TRP transport into the brain may not represent the only factor leading to depressed brain serotonergic activity in NIDDM patients. Other mechanisms might be involved as well, including altered kinetics of blood–brain barrier TRP uptake. Finally, we recognize that factors other than solely brain serotonin, are involved in the pathogenesis of diabetes mellitus-associated hyperphagia. Among these newer putative factors, consistent data suggest that NPY plays a pivotal role.[44] However, it must be noted that the effects of NPY and serotonin on energy intake appear to be closely connected.[45]

In the present study, we also observed a significant reduction of fat intake, which is consistent with previous findings[14] and with animal data suggesting that under specific experimental conditions, increased brain serotonin is involved in reducing fat intake.[46] We acknowledge that the concomitant reduction of carbohydrate and fat intakes may reason against a role for serotonin in modulating macronutrient selection, supporting the thesis that brain serotonin is a non-specific inhibitor of energy intake (for review, see Ref. 15). However, it must be noted that patients receiving 5-HTP reduced their daily energy intake by approximately 1750 kJ/d, and that the inhibition of carbohydrate intake accounted for approximately 75% (−67 g/d, that is, −1100 kJ/d). Patients receiving 5-HTP also reduced their fat intake, but this only accounted for approximately 25% of the cumulative energy intake reduction (−12 g/d, that is, −440 kJ/d). Therefore, it appears reasonable to conclude from the data obtained in the present study, that brain serotonin significantly reduces energy intake, by

predominantly inhibiting carbohydrate intake. Whether the reduction of fat intake is an accompanying, or a serotonin-driven, phenomenon still remains to be ascertained.

The reduction of energy intake in patients receiving 5-HTP was accompanied by a slight, though significant, reduction in body weight. It must be considered, however, that patients were not on a low energy diet, and that the study period was only two-weeks long; a time interval insufficient to obtain a more evident reduction of body weight. The relatively short time of 5-HTP administration may also explain why the metabolic parameters examined, though improved, did not reach statistical significance.

Finally, as reported in previous studies,[13,14] 5-HTP was virtually free from relevant side effects, nausea being the most frequent symptom reported by patients (70% at the end of the first week of treatment). This evidence may raise concern and suggest the possibility that nausea might play a role in the reduction of energy intake and body weight. However, nausea was an episodic and transient symptom, being less frequent during the second week of treatment (only 20% of patients reported its presence), when the reduction in energy intake was still statistically significant. Furthermore, when present, nausea was mild and never caused a patient to stop eating. In one patient only, nausea occurred during the first day of treatment and was so severe he had to withdraw from the study, thus suggesting the subject's hypersensitivity to the drug.

Conclusion

In summary, the data obtained in the present study show that in NIDDM patients, brain tryptophan availability is reduced, possibly contributing to the development of hyperphagia and disturbed eating behaviour. The oral administration of a precursor of brain serotonin, 5-HTP, to these patients is effective in reducing both energy intake, by predominantly inhibiting the intake of carbohydrate, and body weight. These results, when confirmed in a larger sample of patients for a longer period of time, may support the use of 5-HTP in NIDDM patients to achieve a better metabolic control, and delay the onset of major complications.

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