# Plasma Met-Enkephalin Levels in Diabetic Patients: Influence of Autonomic Neuropathy

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**The presence of opioid peptides within pancreatic islets in several animal species and in humans suggests that these peptides could play a role in pancreatic endocrine secretion, influencing glucose metabolism. We measured plasma met-enkephalin (met-Enk) levels in eight neuropathic (four with insulin-dependent diabetes mellitus [IDDM] and four with non–insulin-dependent diabetes mellitus [NIDDM]) and eight nonneuropathic (four IDDM and four NIDDM) diabetic patients to study met-Enk secretion in diabetic patients with asymptomatic autonomic neuropathy. Plasma met-Enk levels were significantly lower in neuropathic compared with nonneuropathic patients both in the IDDM group (28.7 ± 4.8 *v* 61.6 ± 4.1 pg/mL, $P < .0025$) and in the NIDDM group (26.5 ± 3.6 *v* 44.3 ± 4.6 pg/mL, $P < .0125$). This study suggests that the presence of neuropathy in diabetic patients, even if asymptomatic, is associated with a significant decrease of plasma met-Enk levels, thus contributing to a worsening of metabolic control under stress conditions.**


CHANGES in either glucose or insulin levels within the central nervous system have been reported to trigger a number of reflexes that influence plasma glucose and free fatty acid levels, hepatic enzymes involved in glycogen synthesis and gluconeogenesis, secretion of metabolic hormones, and food intake.[1] Commensurate with the brain's influence on the pancreas, the islets of Langerhans are among the most highly innervated endocrine tissues. These nerve trunks contain sympathetic postganglionic fibers originating within the celiac ganglion and parasympathetic preganglionic fibers of the vagus nerve.[2,3] Axons may form a plexus surrounding the islet, with small bundles of axons entering the islet in association with Schwann cells. Individual axons make junctional connections with islet secretory cells[2] containing acetylcholine or norepinephrine, and are associated with the parasympathetic and sympathetic autonomic nervous system. The islets in most species, including humans, contain axons with larger secretory vesicles typical of adrenergic and cholinergic fibers; in mammals, these larger vesicles are thought to contain peptides.[4-6] A large and expanding list of peptides are known to exist within peripheral autonomic nerves, and of these, vasointestinal peptide, cholecystokinin, gastrin-releasing peptide, galanin, neuropeptide Y, calcitonin gene–related peptide, substance P, and enkephalin have been found in the pancreatic nerves.[4,7] Thus, cells of the islets of Langerhans represent a functional syncytium receiving a rich nerve supply including cholinergic, adrenergic, and peptidergic fibers and influencing the various secretory cells. The latter, in turn, are themselves functionally interconnected.

The presence of opioid peptides within pancreatic islets of several animal species, including humans,[8,9] suggests that these peptides may also play a role in pancreatic endocrine secretion and therefore influence peripheral glucose metabolism. On the other hand, they may have a direct peripheral effect on glucose homeostasis.

Studies investigating changes in circulating opioid levels in diabetes mellitus have yielded discordant results reflecting the heterogeneity of this disease. In type II (non–insulin-dependent) diabetes mellitus (NIDDM), β-endorphin (β-End) plasma levels have been found to be elevated,[10] unchanged,[11] or decreased.[12] In insulin-treated type II diabetics, increased plasma β-End levels have been reported.[13] By contrast, patients with type I (insulin-dependent) diabetes mellitus (IDDM) do not show any significant change in β-End plasma levels,[10] whereas very high plasma met-Enk (met-Enk) levels have been recently reported by our group.[14] We hypothesized a negative feedback between met-Enk and insulin secretion similar to that previously reported in genetically obese diabetic mice by Timmers et al.[15]

In addition, counterregulatory mechanisms appear to be impaired in diabetic patients,[16-18] which could influence circulating met-Enk levels. By contrast, since the presence of diabetic neuropathy, especially autonomic neuropathy, could directly or indirectly affect opioid secretion in the bloodstream, we investigated met-Enk plasma levels in type I and type II diabetic patients with or without asymptomatic autonomic neuropathy, excluding patients with a more severe neuropathy where a longstanding impairment of the nervous system may cancel the influence of the nervous system on opioid secretion.

## SUBJECTS AND METHODS

### *Patients*

Sixteen (eight IDDM and eight NIDDM) chronically diabetic patients (nine women and seven men) were studied; eight suffered from neuropathy (four IDDM and four NIDDM) and eight did not (four IDDM and four NIDDM). The mean age was 54.3 ± 2.2 years and diabetes duration 18.3 ± 1.9 years. The mean age and duration of diabetes were 57.2 ± 2.7 years (range, 45 to 63) and 19.5 ± 3.4 years (range, 2 to 36) in neuropathic patients and 51.4 ± 3.5 (range, 35 to 64) and 17.1 ± 1.9 (range, 10 to 26) in nonneuropathic patients, respectively (p = NS). Metabolic control, as assessed by blood glucose levels (174 ± 10 *v* 126 ± 15 mg/dL) and hemoglobin $A_{1c}$ ($HbA_{1c}$) values (8.9% ± 0.55% *v* 7.2% ± 0.44%), was significantly worst in neuropathic versus nonneuropathic diabetics. The mean daily insulin dosage was 50 ±

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7 IU in IDDM subjects. NIDDM patients were all treated with hypoglycemic agents, seven with glibenclamide and one with glibenclamide plus phenformin. Detailed clinical data for these subjects are reported in Table 1.

As control group, we studied 21 normal volunteers (11 men and 10 men) with a mean age of 48 ± 7 years.

In neuropathic and nonneuropathic diabetic patients, plasma met-Enk levels were measured under basal conditions, after a 1-hour rest in the fasting state in the supine position at 8 AM, and are reported as picograms per milliliter.

### *Evaluation of Neuropathy*

Diagnosis of asymptomatic diabetic neuropathy was based on clinical and laboratory examination of the nervous system. Clinical examination was performed at the bedside, and an anamnestic scoring system was used.

The following tests were performed: quantitative sensory testing (Biothesiometer); cardiovascular autonomic tests (postural hypotension, deep breathing, lying-to-standing, and Valsalva maneuver); and unilateral nerve conduction evaluation (electrophysiological examination including peroneal and ulnar motor nerve conduction velocity [MNCV] and median and sural sensory nerve conduction velocity [SNCV]).

Diagnosis of asymptomatic diabetic neuropathy was based on an absence of pain but impaired MNCV or SNCV with or without impairment on Biothesiometer results and impairment shown on at least two cardiovascular autonomic tests.

The quantitative sensory test (Biothesiometer, Biomedical Instrument Co, Newbury, OH) was considered abnormal when the values obtained were higher than those reported by Bax et al[19] (Italian Group of Neuropathy), according to Dyck et al.[20] Postural hypotension was defined as abnormal when systolic blood pressure decreased more than 30 mm Hg at 1 minute after standing. Heart rate variation during the deep-breathing test was defined as altered when it was not greater than 10 beats per minute. The lying-to-standing test was considered abnormal when the electrocardiographic 30th to 15th beat ratio was not greater than 1.01. The Valsalva ratio was defined as abnormal when it was not greater than 1.1. Finally, nerve conduction velocity (NCV) of median, ulnar, peroneal, and sural nerves was defined as abnormal when it was less than 44.9, less than 48.0, less than 42.4, and less than 42.0 m/s, respectively.

### *Assay of Met-Enk*

EDTA blood samples were collected in glass tubes on ice and immediately centrifuged in a refrigerated centrifuge for 15 minutes at 1,500 rpm. Met-Enk was extracted from plasma on ODS-SILICA columns and measured by radioimmunoassay using a commercially available kit (ImmunoNuclear, Stillwater, MN) as described elsewhere.[14]

Assay sensitivity was 5 fmol; interassay and intraassay coefficients of variation were 9.5% ± 0.5% and 6.8% ± 0.7%, respectively, at 50% binding. The affinity constant of the antibody for met-Enk is $4 \times 10^{-12}$ pmol/L. The percentage cross-reactivity for leu-enkephalin is 2.8% and for other peptides less than 0.002%.

### *Statistical Analysis*

Statistical analysis for comparison of met-Enk values in different groups of patients was performed using Student's $t$ test for unpaired data.

## RESULTS

Plasma met-Enk levels in normals were 31.5 ± 5.7 pg/mL (mean ± SEM).

There were no sex differences in plasma met-Enk levels among diabetic patients (women 40 ± 6 pg/mL *v* men 41 ± 5.6).

Diabetic patients with neuropathy did not differ from nonneuropathic diabetic patients in age and diabetes duration, whereas they differed significantly in metabolic control ($HbA_{1c}$, 8.9% ± 0.5% *v* 7.2% ± 0.4%, $P < .01$; fasting blood glucose, 174 ± 10 *v* 126 ± 15 mg/dL, $P < .01$). In addition, age, diabetes duration, and metabolic control ($HbA_{1c}$) were similar in IDDM and NIDDM patients as a group (both neuropathic and nonneuropathic). On the other hand, in the IDDM group, $HbA_{1c}$ was significantly higher in neuropathic than in nonneuropathic patients (8.8% ± 1.1% *v* 6.8% ± 0.4%, $P < .025$); similarly, in the NIDDM group, $HbA_{1c}$ was significantly higher in neuropathic than in nonneuropathic patients (8.9% ± 0.4% *v* 7.6% ± 0.8%, $P < .025$) (Table 2).

The presence of neuropathy in diabetic patients as a group was associated with significantly lower plasma met-Enk levels than in nonneuropathic diabetic patients (27.6 ± 2.8 *v* 52.9 ± 4.3 pg/mL, $P < .005$). In addition, the presence of diabetic neuropathy was associated with a significant decrease of plasma met-Enk levels in both IDDM (28.7 ± 4.8 *v* 61.6 ± 4.1 pg/mL, $P < .0025$) and NIDDM (26.5 ± 3.6 *v* 44.3 ± 4.6 pg/mL, $P < .0125$) patients. Finally, plasma met-Enk levels among nonneuropathic patients were significantly higher in IDDM than in NIDDM patients (61.6 ± 4.1 *v* 44.3 ± 4.6 pg/mL, $P < .025$) (Table 2).

**Table 1. Clinical Findings in the Diabetic Patients**

| Group | Age (yr) | Sex Distribution | Diabetes Duration (yr) | BMI | Therapy (insulin [IU/d] or OHA) |
|---|---|---|---|---|---|
| IDDM and NIDDM | 54.3 ± 2.2 | 9F/7M | 18.3 ± 1.9 | 25.7 ± 0.6 | — |
| 1. Nonneuropathic | 51.4 ± 3.5 | 5F/3M | 17.1 ± 1.9 | 25.2 ± 0.9 | — |
| 2. Neuropathic | 57.2 ± 2.7 | 4F/4M | 19.5 ± 3.4 | 26.2 ± 0.8 | — |
| IDDM | 51.4 ± 3.9 | 4F/4M | 20.2 ± 2.9 | 24.6 ± 0.8 | 50 ± 7 |
| 3. Nonneuropathic | 44.8 ± 4.7 | 2F/2M | 14.2 ± 1.9* | 22.8 ± 0.1 | 38 ± 2† |
| 4. Neuropathic | 58.0 ± 4.3 | 2F/2M | 26.2 ± 3.3 | 25.4 ± 1.0 | 61 ± 12 |
| NIDDM | 57.2 ± 2.0 | 5F/3M | 16.4 ± 2.6 | 27.0 ± 0.4 | OHA |
| 5. Nonneuropathic | 58.0 ± 2.1 | 2F/2M | 20 ± 2.9* | 26.5 ± 0.4 | OHA |
| 6. Neuropathic | 56.5 ± 3.9 | 2F/2M | 12.7 ± 3.7 | 27.7 ± 0.2 | OHA |

NOTE. Data are the mean ± SEM.

Abbreviation: OHA, oral hypoglycemic agent.

*$P < .001$, †$P < .05$: *v* neuropathic patients.

**Table 2. Metabolic Findings in the Diabetic Patients**

| Group | $HbA_{1c}$ (%) | BG (mg/dL) | Met-Enk (pg/mL) |
|---|---|---|---|
| IDDM and NIDDM | 8.0 ± 0.4 | 150 ± 11 | 40.3 ± 4.1 |
| 1. Nonneuropathic | 7.2 ± 0.4† | 126 ± 15† | 52.9 ± 4.3 |
| 2. Neuropathic | 8.9 ± 0.5 | 174 ± 10 | 27.6 ± 2.8 |
| IDDM | 7.8 ± 0.7 | 162 ± 14 | 45.1 ± 6.9 |
| 3. Nonneuropathic | 6.8 ± 0.4* | 147 ± 27 | 61.6 ± 4.1 |
| 4. Neuropathic | 8.8 ± 1.1 | 177 ± 2 | 28.7 ± 4.8 |
| NIDDM | 8.3 ± 0.5 | 138 ± 16 | 35.4 ± 4.3 |
| 5. Nonneuropathic | 7.6 ± 0.8* | 105 ± 7* | 44.3 ± 4.6 |
| 6. Neuropathic | 8.9 ± 0.4 | 172 ± 21 | 26.5 ± 3.6 |

NOTE. Data are the mean ± SEM.

Abbreviation: BG, blood glucose.

*$P < .025$, †$P < .01$: *v* neuropathic patients. Met-Enk statistical comparison: 1 *v* 2, $P < .005$; 3 *v* 4, $P < .0025$; 5 *v* 6, $P < .0125$; 3 *v* 5, $P < .025$.

## DISCUSSION

We have shown herein that plasma met-Enk levels are significantly higher in nonneuropathic diabetics (IDDM and/or NIDDM) compared with normal subjects, confirming our previous results in IDDM patients.[13]

Among diabetic patients with a well-characterized degree of initial autonomic neuropathy, plasma met-Enk levels are significantly higher in nonneuropathic IDDM patients compared with nonneuropathic NIDDM patients, despite younger age, shorter diabetes duration, and similar metabolic control. These results suggest that in nonneuropathic diabetics, met-Enk values are not age-related, and are likely related, above all, to insulin deficiency and impaired glucose metabolism.

By contrast, the presence of neuropathy in diabetic patients (both IDDM and NIDDM), although asymptomatic, is associated with a significant reduction of met-Enk compared with the levels found in nonneuropathic diabetic patients.

It has been suggested that the increase of met-Enk secretion in diabetics could be related to sympathetic hyperactivity as a consequence of impaired metabolic control. We suggest that the occurrence of diabetic neuropathy limits the increase of met-Enk levels.

Our findings are in agreement with current knowledge that poor metabolic control is associated with an increase of counterregulatory hormones and especially of sympathetic activity.[21-22]

Therefore, the increase in met-Enk levels in diabetes could be a direct consequence of an impaired feedback between insulin and enkephalin or an indirect consequence of an increase of catecholamines, since epinephrine and enkephalins are released simultaneously in the bloodstream.[23] Since plasma catecholamine levels were not measured in this study, further studies are needed to assess this possibility.

In addition, experimental studies have demonstrated that hypothalamic and pituitary β-End and pituitary met-Enk levels are significantly decreased in rats with streptozotocin- or alloxan-induced diabetes.[24]

In these rats, a reduction of myelin fibers and axon size has also been observed, and these morphologic abnormalities could induce more pronounced structural changes including axonal loss and segmental demyelination. Insulin replacement therapy in experimental diabetes reversed the reduction of both opioids, suggesting the importance of insulin and glucose metabolism in the regulation of met-Enk and β-End synthesis.[24]

In our study, diabetic patients with asymptomatic neuropathy showed a poorer metabolic control compared with nonneuropathic patients. Therefore, we cannot establish which of the parameters (impaired glucose metabolism and/or neuropathy) had more influence on the opioid system. Further studies are required, particularly to clarify the role of an impairment of the nervous system on the opioid system and the significance of a reduction in met-Enk on metabolism and stress responses in diabetic patients.

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