



Long-Acting C-Peptide and Neuropathy in Type 1 Diabetes: A 12-Month Clinical Trial

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OBJECTIVE

Lack of C-peptide in type 1 diabetes may be an important contributing factor in the development of microvascular complications. Replacement of native C-peptide has been shown to exert a beneficial influence on peripheral nerve function in type 1 diabetes. The aim of this study was to evaluate the efficacy and safety of a long-acting C-peptide in subjects with type 1 diabetes and mild to moderate peripheral neuropathy.

RESEARCH DESIGN AND METHODS

A total of 250 patients with type 1 diabetes and peripheral neuropathy received long-acting (pegylated) C-peptide in weekly dosages of 0.8 mg ($n = 71$) or 2.4 mg ($n = 73$) or placebo ($n = 106$) for 52 weeks. Bilateral sural nerve conduction velocity (SNCV) and vibration perception threshold (VPT) on the great toe were measured on two occasions at baseline, at 26 weeks, and at 52 weeks. The modified Toronto Clinical Neuropathy Score (mTCNS) was used to grade the peripheral neuropathy.

RESULTS

Plasma C-peptide rose during the study to 1.8–2.2 nmol/L (low dose) and to 5.6–6.8 nmol/L (high dose). After 52 weeks, SNCV had increased by 1.0 ± 0.24 m/s ($P < 0.001$ within group) in patients receiving C-peptide (combined groups), but the corresponding value for the placebo group was 1.2 ± 0.29 m/s. Compared with basal, VPT had improved by 25% after 52 weeks of C-peptide therapy (Δ for combined C-peptide groups: -4.5 ± 1.0 μ m, placebo group: -0.1 ± 0.9 μ m; $P < 0.001$). mTCNS was unchanged during the study.

CONCLUSIONS

Once-weekly subcutaneous administration of long-acting C-peptide for 52 weeks did not improve SNCV, other electrophysiological variables, or mTCNS but resulted in marked improvement of VPT compared with placebo.

C-peptide, an integral component of the insulin biosynthesis, is the 31-amino acid peptide that makes up the connecting segment between the parts of the proinsulin molecule that become the A and B chains of insulin. It is split off from proinsulin and secreted together with insulin in equimolar amounts. Much new information on C-peptide physiology has appeared during the past 20 years; for an overview, see Wahren et al. (1). C-peptide has been shown to bind specifically to cell membranes (2) and elicit intracellular signaling via G-protein- and Ca^{2+} -dependent pathways (3,4), resulting in activation and increased expression of endothelial nitric oxide (5), Na^+ , K^+ -ATPase (6), and several transcription factors of importance for antioxidative,

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anti-inflammatory, and cell-protective reactions (7,8). Studies in animal models of diabetes and early clinical trials in patients with type 1 diabetes (T1DM) demonstrate that C-peptide in physiological replacement doses elicits beneficial effects on early stages of diabetes-induced functional and structural abnormalities of the peripheral nerves, the autonomic nervous system, and the kidneys (9). Even though much is still to be learned about C-peptide and its mechanism of action, the available evidence presents the picture of a bioactive peptide with therapeutic potential.

Several studies have demonstrated that C-peptide administration to animals with experimental diabetes and neuropathy is accompanied by a reversal of diabetes-induced slowing of nerve conduction velocity (NCV) and amelioration of nerve structural abnormalities (10,11). It has been suggested that the beneficial action of C-peptide is related to improved endoneurial blood flow (11,12), increased levels of nerve Na^+ and K^+ -ATPase (10), and stimulation of several transcription factors regulating cytoprotective, antiapoptotic, and anti-inflammatory cellular effects (7,8). Two clinical trials involving C-peptide and subjects with T1DM with impaired nerve function have been reported. The 49 patients in the first study showed slowing of sural NCV (SNCV) at baseline but no clinical signs or symptoms of overt neuropathy. After 3 months of replacement C-peptide treatment together with the patients' regular insulin therapy, the average improvement of SNCV above placebo treatment was 2.1 m/s (13). A subsequent study in 139 patients with T1DM with manifest peripheral neuropathy indicated that C-peptide treatment for 6 months resulted in improved SNCV, signs of reduced vibration perception threshold (VPT), and amelioration of clinical signs and symptoms as evaluated by a clinical neuropathy assessment score (14).

The biological half-life of C-peptide in healthy subjects is ~ 30 min and longer in patients with T1DM (15). In clinical trials aiming to evaluate the effects of replacement of C-peptide in subjects with T1DM, administering the peptide 4–5 times daily has therefore been necessary to maintain its plasma concentration within the normal range for as long as possible (13,14). A long-acting form of C-peptide has now been developed

to improve C-peptide exposure and patient convenience. The modified molecule comprises human C-peptide that has been covalently bound at its N-terminus to a branched 40-kDa polyethylene glycol (PEG) molecule (16). In vitro cell based studies have demonstrated that PEG-C-peptide retains the bioactivity of the native peptide (16). Measurements of NCV in streptozotocin-induced diabetic rats show that the PEG-C-peptide and the native peptide are approximately equipotent with regard to their ability to ameliorate the diabetes-induced slowing of NCV (17). Studies in subjects with T1DM have shown that the PEG-C-peptide can be administered without adverse events and that its biological half-life is ~ 6 –7 days (18).

The current study was undertaken to evaluate the long-term efficacy and safety of PEG-C-peptide in patients with T1DM with mild to moderate peripheral neuropathy.

RESEARCH DESIGN AND METHODS

Patient Material

Subjects with T1DM and mild to moderate diabetic peripheral neuropathy (DPN) were screened with the following inclusion criteria: 18 to 65 years of age inclusive; T1DM for a minimum of 4 years, with stable diabetic regimen (for at least 3 months) that provided adequate glucose control, otherwise in good general health; serum creatinine ≤ 1.5 mg/dL (≤ 133 $\mu\text{mol/L}$); no concomitant medication that could potentially influence peripheral nerve function or presence of clinical signs or symptoms (prespecified) of DPN at screening; presence of bilateral, recordable sural sensory nerve responses consistent with mild to moderate DPN, that is, bilateral SNCV at least 2 SD below the mean corrected for age (18–40 years: ≤ 48 m/s, 41–60 years: ≤ 46.5 m/s, and >60 years: ≤ 44 m/s) and minimum sural nerve action potential amplitude (SNAP) of 2 μV on at least two occasions during screening; C-peptide deficient with a fasting concentration of <0.1 nmol/L; and BMI ≥ 18.0 and <35.0 kg/m².

Study Design and Procedure

This was a multicenter, phase 2b, randomized, double-blind, placebo-controlled, parallel-group study. The study screened 756 subjects and enrolled 250 at 32 clinical sites in the U.S. ($n = 23$), Canada ($n = 2$),

and Sweden ($n = 7$). Subjects were randomized in a 2:2:3 ratio into one of three treatment groups: group 1, high-dose PEG-C-peptide (2.4 mg, $n = 73$ subjects); group 2, low-dose PEG-C-peptide (0.8 mg, $n = 71$ subjects); or group 3, placebo (vehicle, 10 mmol/L sodium phosphate buffer with 4.7% sorbitol, $n = 106$ subjects). For details, see Supplementary Fig. 1. The randomization was stratified by the baseline glycosylated HbA_{1c} value (≤ 8 or $>8\%$), duration since T1DM diagnosis (≤ 15 or >15 years), and geographic region (North America or Sweden).

The study protocol was approved by the regional human ethics committees. All patients were informed of the nature, purpose, and possible risks of the study before consenting to participate. After giving their informed consent, patients underwent a physical examination, including electrocardiogram, measurement of blood pressure, and clinical chemistry laboratory testing. In addition, peripheral venous blood samples were collected predose on day 0 and postdose at weeks 4, 16, 26, and 52, or at early termination (ET), to determine PEG-C-peptide concentrations and for population pharmacokinetic analysis. Neurological and neurophysiological examinations were also performed, as described below.

Subjects participated in the study for ~ 13 months. This included a 4-week screening period, a 52-week treatment period during which weekly subcutaneous doses of 0.8 mg PEG-C-peptide, 2.4 mg PEG-C-peptide, or placebo were self-administered, and a safety follow-up visit ~ 1 week after the last dose. The study drug was supplied by Cebix Inc. (San Diego, CA) as a sterile aqueous solution containing PEG-C-peptide in 0.5-mL filled vials at two concentrations (2.7 mg/mL for the low-dose and 8.0 mg/mL for the high-dose treatment groups) and placebo designed for subcutaneous administration.

Neurophysiological Assessments

Bilateral sural sensory (SNCV) and peroneal motor (MNCV) nerve conduction measurements were performed in duplicate on two occasions between screening and day 0 (on 2 different days within 14 days of each other), at week 26, and at week 52 or at ET. A third, repeat NCV assessment was performed if a technical error was present in the recording or if

the variability of the duplicate measures of NCV from the same nerve on the same side for a single time point was greater than 12%. Measurements were performed as described earlier (19). Briefly, for the sural sensory nerve (antidromic), initial (SNCV_i) and peak (SNCV_p) conduction velocity of the distal segment (lower calf to below ankle) and amplitude of compound sensory response (SNAP, initial depolarization at ankle, measured baseline to peak) were measured. Surface electrodes were placed on cleaned skin with a conducting medium between the electrode and the skin. Skin temperature was recorded at the start and end of electrophysiological testing for each side and maintained at values $\geq 31.0^{\circ}\text{C}$ throughout testing. Subjects were warmed (if necessary) using a hydrocollator (circulating warm water pad), warmed towels, or a temperature-controlled blanket wrap. For the peroneal motor nerve (orthodromic), MNCV of the knee-to-ankle segment and amplitude of compound motor response (initial depolarization after stimulation at the ankle and fibular head, measured baseline to peak) were measured. Results from all nerve conduction studies were reviewed, rescored, and approved by a central neurophysiology laboratory.

Determination of VPT of the lower limbs at the great toe was performed bilaterally on two occasions between screening and day 0, at week 26, and at week 52 (or ET) using a Vibratron II instrument (Physitemp Instruments, Clifton, NJ). A forced-choice algorithm was used to determine VPT at the great toe as described previously (20). Results from all VPT studies were reviewed and approved by the central neurophysiology laboratory.

Neurological Examinations and Clinical Symptom Assessments

The modified Toronto Clinical Neuropathy Score (mTCNS) (21) was used to characterize DPN. The assessment was conducted predose at the day 0 visit and postdose administration at the week 26 and 52 (or ET) visits.

Degree of pain intensity due to DPN in the extremities (hands and feet) was rated by the study subjects using an 11-point rating scale (22) at the day 0 visit and postdose administration at the week 26 and 52 (or ET) visits.

Sexual function questionnaires were provided to the study subjects for completion predose at the day 0 visit and at the week 26 and 52 (or ET) visits. Male subjects completed the International Index of Erectile Function (IIEF) questionnaire (23). Quality of life was assessed by the Neuro-QoL questionnaire (24), provided to the study subjects for completion on day 0 and at week 52 (or ET).

Analyses

Hematology, clinical chemistry, and urinalysis testing were done throughout the study at screening, predose on day 0, and postdose at weeks 4, 8, 16, 26, 39, and 52 (or ET). Routine clinical laboratory tests including HbA_{1c} were determined by standard procedures and conducted by a central laboratory. The PEG-C-peptide concentration in plasma was assayed using a validated quantitative sandwich ELISA. Briefly, a monoclonal antibody specific for C-peptide was precoated onto a microplate, standards and samples were pipetted into the wells, and PEG-C-peptide was captured by the immobilized antibody. Any unbound substances were washed away, and an enzyme-linked monoclonal antibody specific for a different site on the C-peptide was added to the wells. After a wash to remove any unbound antibody-enzyme conjugate, a substrate solution was added to the wells, and the developed color, measured at 450 nm by a microplate reader, was proportional to the amount of PEG-C-peptide bound in the initial step. The calibration of the assay ranged from 0.21 to 13.6 nmol/L.

Urine samples were collected during screening, at predose on day 0, at week 26, and at week 52 (or ET) to evaluate change in the quantitative urinary albumin-to-creatinine ratio (UACR). Serum samples were collected during screening and at the week 26 and 52 (or ET) visits to evaluate cystatin C concentrations. Serum C-reactive protein (CRP) concentrations were determined in blood samples collected predose at day 0 and at week 52 (or ET).

Statistical Methods

The study was designed to provide greater than 80% statistical power to detect a change in the primary variable SNCV from baseline to week 52 of 1.0 m/s or greater using an α level of 0.05. All efficacy and safety variables were

analyzed using descriptive statistics, the Student *t* test, Spearman rank correlation test, and an ANCOVA model. Treatment groups, HbA_{1c}, diabetes duration, geographic region, baseline SNAP, and baseline VPT were used as covariates. VPT results were analyzed with exclusion for technical reasons of the findings for one subject in the placebo group. The data presented are based on two-sided tests and the $P < 0.05$ level of significance.

RESULTS

Baseline

The study screened 756 subjects; of these, 250 were subsequently randomized to the low-dose ($n = 71$), the high-dose ($n = 73$), or the placebo group ($n = 106$) and received at least one dose of the study drug (intent to treat [ITT], safety population). Of these, 11 subjects discontinued their participation in the study before any postbaseline SNCV assessment, so that the modified ITT (mITT) population included 239 subjects. The per-protocol population (PP) excluded an additional 41 subjects due to adverse events, withdrawal of consent by the subject, insufficient compliance ($<80\%$) with the dosing regimen, or subjects lost to follow-up. The PP therefore included 198 subjects; for details of treatment groups and subject disposition, see Supplementary Fig. 1.

For the mITT population, there were no significant differences among the study groups with regard to age (range of means for the groups: 45.7–47.1 years), sex distribution (50.0–63.4% male subjects), BMI (26.0–26.5 kg/m²), or duration of T1DM (26.7–27.3 years). Likewise, blood glucose (9.0–9.7 mmol/L), HbA_{1c} (7.8–7.9% [61.7–62.8 mmol/mol]), daily insulin requirements, and blood pressure were similar in the groups. Blood chemistry results were unremarkable, and the plasma C-peptide levels were low (0.031–0.036 nmol/L), as expected.

The electrophysiological measurements at baseline indicated significant slowing of SNCV_i (40.9–41.4 m/s) and MNCV (40.7–41.9 m/s) and reduction of SNAP (6.8–7.9 μV), compatible with mild to moderate diabetes-induced functional nerve impairment of similar magnitude in the three treatment groups. VPT was markedly elevated (indicating impairment) and of similar

magnitude in all three groups (16.5–21.0 μm). Univariate analysis showed that VPT was negatively correlated to baseline SNCV_i ($r = 0.34$, $P < 0.001$), SNAP ($r = 0.51$, $P < 0.001$), and diabetes duration ($r = 0.26$, $P < 0.001$). The range between treatment groups for the mTCNS score was 4.8–5.3, and pain intensity ratings were 1.1–1.3 for the different groups at baseline. The erectile function score, assessed by the IIEF index, was 18.7–20.5 at baseline. Glomerular filtration rates, estimated from the plasma concentrations of cystatin C, were within the normal range and similar in the study groups (115–120 mL/min/1.73 m²). The UACR in subjects with microalbuminuria at baseline was 0.70–0.83 mg/mmol in the groups. CRP was similar in the groups (1.8–2.2 mg/L). Details of the baseline data for the mITT population and the three treatment groups are presented in Supplementary Table 1.

Study Outcome

During the course of the 12-month study period, there were no significant changes in fasting blood glucose. Levels of HbA_{1c} remained stable and varied within the treatment groups on average less than 0.1% (0.9 mmol/mol) between baseline and 52 weeks. The daily insulin requirements tended to decrease slightly during the study; the decrease was not different among the groups but was most marked in the high-dose group (-7% , $P < 0.05$ within group). Plasma concentrations of PEG-C-peptide, illustrated in Fig. 1, varied in the low-dose group between 1.8 and 2.2 nmol/L during the study, and the concentration range for the high-dose group was 5.6–6.8 nmol/L during the study.

Table 1 summarizes the results for the neurophysiological variables in the mITT population. SNCV_i had increased after 52 weeks by 1.30 ± 0.41 m/s in the low-dose group ($P < 0.005$ within group) and by 0.64 ± 0.27 m/s in the high-dose group ($P < 0.025$ within group). The difference versus placebo-treated subjects was not significant because, surprisingly, an SNCV_i improvement of similar magnitude (1.19 ± 0.29 m/s, $P < 0.001$ within group) was observed in the placebo group. Similar results were found for SNCV_p. Multiple regression analysis showed that the

increase in SNCV_i during the study was negatively and independently related to both SNAP ($P < 0.001$) and SNCV_i ($P < 0.01$) at baseline. This was true for the group receiving the PEG-C-peptide and the placebo subjects. SNAP and MNCV did not change significantly during the study in either study group. Analysis of the findings in the PP population yielded similar results. Assessment of the change in SNCV_i during the study using ANCOVA or examination of subgroups based on age, duration of T1DM, estimated severity of neuropathy, or glycemic control did not result in different findings.

There was a gradual lowering of VPT, indicating improvement in subjects receiving PEG-C-peptide, as demonstrated in Fig. 2, for the mITT population. The reduction in VPT attained statistical significance versus placebo after 52 weeks for both groups (low dose: $P < 0.003$, high dose: $P < 0.02$). Thus after 52 weeks, subjects in the low-dose group had lowered their VPT by an average of 31% compared with baseline; the corresponding value for the high-dose group was 19%. VPT improved between 26 and 52 weeks ($P < 0.025$) in the low-dose group. The difference in VPT response between the dose groups did not attain statistical significance. In contrast to the SNCV results, VPT in the placebo group changed very little from baseline during the study (Fig. 2). Multiple regression analysis indicated significant negative correlations to baseline VPT, SNAP, and body height. ANCOVA analysis confirmed the above findings, with the exception that change in VPT for the high-dose versus placebo group did not reach statistical significance. Finally, analysis of results for the ITT population confirmed statistically significant improvements in VPT versus placebo for the low-dose group (-28% , $P < 0.003$) and the high-dose group (17% , $P < 0.02$) after 12 months.

The mTCNS, pain, and sexual function scores did not change significantly during the study nor did subgroup analysis involving the subjects most affected at baseline reveal significant differences between subjects treated with PEG-C-peptide or placebo subjects. Glomerular filtration rates increased slightly in the PEG-C-peptide- and placebo-treated subjects (3–4%, $P < 0.02$ – 0.001 within groups). The UACR in the 24 subjects that showed microalbuminuria at baseline

tended to decrease, but not significantly so in either treatment group. Finally, CRP remained unchanged in PEG-C-peptide-treated subjects during the study but increased in those receiving placebo (85%); this was, however, the result of >30 mg/L increments in CRP in four subjects in the placebo group. After elimination of the outliers, no difference remained between placebo-treated and PEG-C-peptide-treated subjects with regard to CRP.

Evaluation of the safety population showed that PEG-C-peptide was well tolerated and that there was a low and similar incidence of treatment-related adverse events (11.3–16.4%) in all three treatment groups; the most common were nasopharyngitis, upper respiratory tract infection, and nausea. Nonsevere hypoglycemic events occurred in 66–74% of the subjects in the different treatment groups, and severe hypoglycemic events (subject requiring the assistance of another person) were observed in 0–4% of the subjects in the groups. One such event was assessed as treatment related; this subject was in the placebo group. There were no notable laboratory results or clinically observable changes in vital signs over time. Minor electrocardiogram changes (PR interval shifts) occurred in a similar percentage in the treatment groups. Most subjects had negative anti-PEG-C-peptide antibody results.

CONCLUSIONS

The observed improvements in SNCV_i during the 12-month study period in subjects receiving PEG-C-peptide were significant within both treatment groups. However, a similar improvement was also observed in the placebo group. The differences in SNCV response for subjects treated with PEG-C-peptide or placebo were not statistically different, and the study thus failed to meet its primary end point. The background to the unexpected improvement in the placebo group is not apparent. The natural progression of DPN would be expected to result in a slowing of SNCV_i by ~ 0.6 – 0.7 m/s after 12 months (25,26). Nevertheless, several clinical trials in patients with diabetic neuropathy have reported increases in SNCV_i in the placebo group of a similar or somewhat smaller magnitude (14,27,28) than that observed in the current study.

PEG-C-Peptide Plasma Concentrations

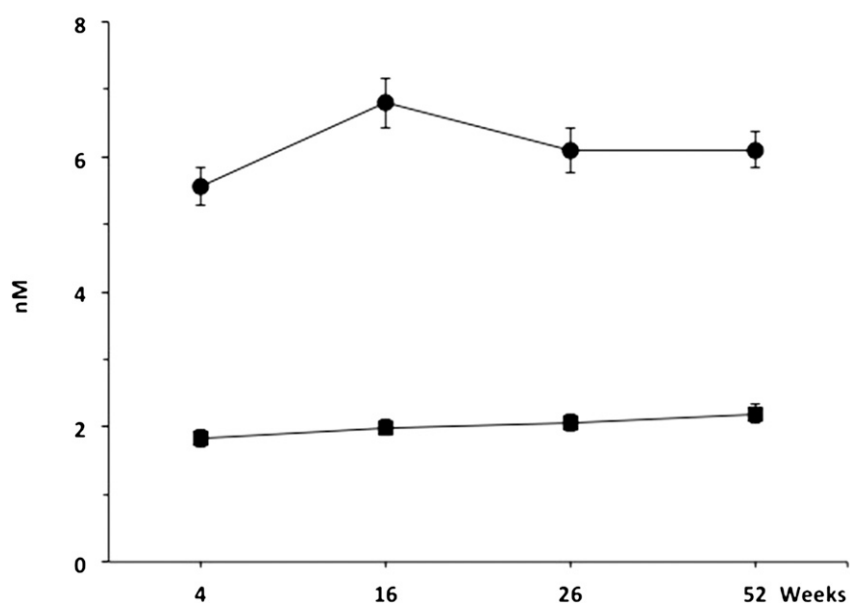


Figure 1—Plasma concentration of PEG-C-peptide after 4, 6, 9, and 12 months of the study in the subjects receiving weekly dosages of 0.8 mg (■) and 2.4 mg (●).

A multivariate analysis of the clinical factors that might contribute to this effect (29) has indicated that decreases in HbA_{1c} and plasma triglyceride concentration, in particular those that occur within 2 months after onset of the study, may be associated with improvements in SNCV_i. In the current study, however, patients receiving placebo showed unchanged HbA_{1c} values at 6 months and a 0.1% increase after 12 months. Likewise, plasma triglyceride levels were unchanged at 6 and 12 months compared with baseline in the placebo group. In addition, blood pressure values were unchanged at 6 and 12 months in relation to baseline in all treatment groups, suggesting that

other explanations have to be sought for the observed improvement in SNCV_i in the placebo group. It is noted that SNCV limits (<2 SD below normal) were included in the inclusion criteria and that regression of the mean could be a confounding factor. Most patients, however, showed baseline SNCV values that were 3–4 SD below normal (Table 1), indicating that regression of the mean was probably not an important factor in the observed increase in SNCV at the end of the study. The possibility may also be considered that this effect could arise from the extra care and attention afforded participants in the clinical trial and from changes in lifestyle (regular assessment of body

weight, blood pressure, and BMI or changes in diet, smoking habits, etc.) inspired by the study, even though one would not expect such factors to influence an objectively measured variable such as SNCV_i. Irrespective of the mechanism involved, the present and previous findings of a marked placebo response for SNCV in clinical trials of diabetic neuropathy raises the question whether NCV is an optimal and biologically meaningful outcome variable in studies of this type.

A striking finding in the current study is the observation of a progressive improvement in VPT during the 12-month treatment with PEG-C-peptide (Fig. 2), despite nonsignificant changes in SNCV. This finding may reflect differences in the mechanisms of conduction versus transduction of neural impulses. Changes in transduction reflect membrane receptor characteristics limited to the distal extreme of specific subtypes of sensory axons. In the case of vibration, the principal receptor is Pacinian corpuscles in the skin that are innervated by A β fibers. Transduction takes place uniquely at the distal extreme of the axon and is largely influenced by the integrity of this limited segment. Studies have documented that the initial effect of toxic neuropathy is a loss of the surface area of the pseudopod extensions of the distal axon within the Pacinian corpuscle and a consequent diminution of transduction (30). In contrast, changes in the speed of conduction are largely a function of factors that influence the elongated tract of the nerve, including the cross-sectional diameter of axons, the degree of myelination, and the integrity of ion clusters at the nodes of Ranvier (31). Thus, it is reasonable that some aspects of distal sensory function may be influenced by a treatment option that has little or no direct effect on nerve conduction velocity. The alternative is the unsupported belief that any intervention in the onset and progression of a sensory neuropathy must alter conduction velocity.

The marked VPT improvement observed in the current study, although associated with nonsignificant changes in SNCV, other electrophysiological variables, or mTCNS, can be interpreted as targeted improvement in a key aspect of sensory function (e.g., the conversion of

Table 1—Neurophysiological and neurological findings at baseline and after 12 months of treatment (mITT population)

	Baseline n = 239	Change from baseline at 12 months			
		Placebo n = 102	PEG-C-peptide		
			Low dose n = 66	High dose n = 71	Both doses n = 137
SNCV _i (m/s)	41.2 \pm 0.2	1.19 \pm 0.29***	1.30 \pm 0.41**	0.64 \pm 0.27*	0.96 \pm 0.24***
SNCV _p (m/s)	33.0 \pm 0.3	1.04 \pm 0.24***	1.10 \pm 0.30**	0.44 \pm 0.20*	0.76 \pm 0.18***
SNAP (μ V)	7.4 \pm 0.3	−0.2 \pm 0.2	0.1 \pm 0.2	0.0 \pm 0.2	0.0 \pm 0.1
MNCV (m/s)	41.2 \pm 0.3	0.3 \pm 0.2	0.3 \pm 0.2	0.2 \pm 0.3	0.3 \pm 0.2
mTCNS	5.0 \pm 0.3	−1.0 \pm 0.3	−1.5 \pm 0.5	0.3 \pm 0.3	−0.9 \pm 0.3

Data are mean \pm SE. The *denote significance of change within group. * P < 0.05; ** P < 0.01; *** P < 0.001.

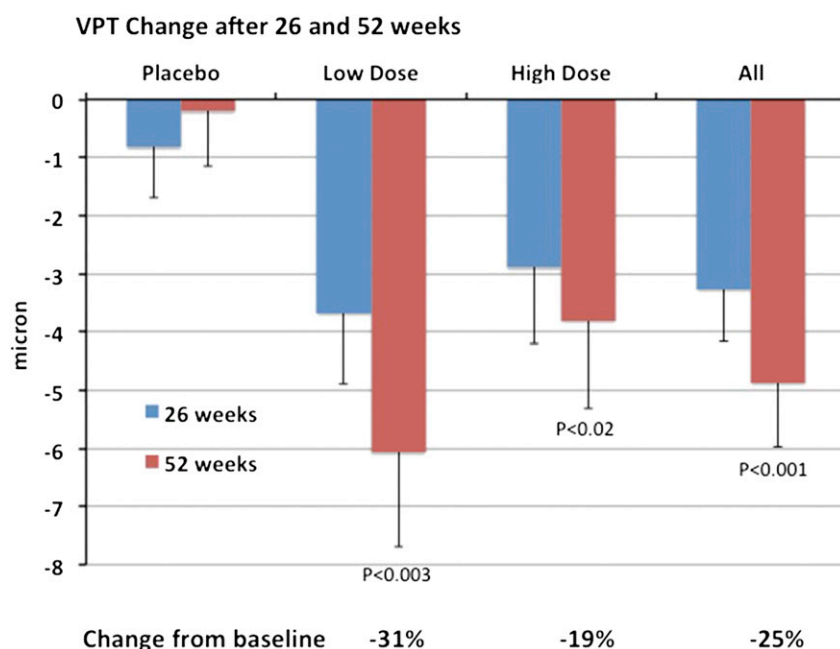


Figure 2—Improvement in VPT in subjects receiving placebo or 0.8 mg per week (low-dose) or 2.4 mg per week (high-dose) of PEG-C-peptide and combined dose groups after 26 and 52 weeks. *P* values denote significance of difference vs. the corresponding value for the placebo group. The improvement in VPT between 26 and 52 weeks in the low-dose group showed $P < 0.05$. Values below the 52 week columns indicate the percent improvement in VPT compared with baseline.

mechanical energy to neural signals—transduction). Two previous clinical trials have both suggested a beneficial effect of native C-peptide on VPT in subjects with T1DM with peripheral neuropathy (12,13). The longer duration of the current study and an improved technique of recording VPT possibly contributed to the present distinctive improvement. Because progressive deficits in sensation are often considered the hallmark of diabetic polyneuropathy, the observed effects of C-peptide in the current study are an important finding.

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Author Contributions. J.W., H.F., M.D., and J.C.A. designed, planned, and conducted the study. J.W. drafted the manuscript. All authors reviewed, revised, and approved the final manuscript. J.W. is the guarantor of this work and, as such, had full access to all the data in the study

and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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