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Original article

Nitrosative stress but not glycemic parameters correlate with improved neuropathy in nonseverely obese diabetic patients after Roux-Y gastric bypass

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Abstract

Background: Diabetic neuropathy is common in type 2 diabetic patients (T2DM) but tight glycemic control does not improve the symptoms. In contrast, Roux-Y gastric bypass (RYGB) has a positive effect on active neuropathic symptoms, independent from glycemic control. The purpose of the present study was to identify potential mechanisms of improved diabetic neuropathic symptoms after RYGB.

Methods: A prospective cohort of 20 patients with insulin-dependent T2DM and BMI <35 kg/m^2 were treated with RYGB. Nineteen patients had complete follow-up. Fasting glucose, HbA1c (glycated hemoglobin), markers for nitrosative, carbonyl, and oxidative stress (nitrotyrosine, carboxylated-lysine (CML), methylglyoxal, oxidized low-density-lipoprotein (oxLDL)) as well as Neuropeptid Y and Neurokinin A were investigated over 12 months. Neuropathy was assessed using the Neuropathy Deficit Score (NDS).

Results: The preoperative NDS improved within twelve months (5.1 ± 0.6 to 2.6 ± 0.4, P = .010). Fasting glucose and HbA1c also improved compared to preoperative values (201.1 ± 16.6 mg/dL to 128 ± 8.7 mg/dL, P = .004 and 8.5 ± 0.3% (53 ± 3.3 mmol/mol) to 7 ± 0.3% (67 ± 3.3 mmol/mol), P = .001, respectively). Nitrotyrosine, CML, and methylglyoxal all 3 decreased postoperatively (1067.3 ± 266.9 nM to 355.8 ± 36.4 nM, P = .003; 257.1 ± 10.2 ng/ml to 215.3 ± 18.3 ng/ml, P = .039; 402.3 ± 3.9 nM to 163.4 ± 10.3 nM, P = .002). OxLDL remained unchanged. Fasting glucose and HbA1c did not correlate with improved neuropathy. The decrease in nitrotyrosine correlated with improvement in the NDS after 6 and twelve months (r = .9, P < .001 and r = .68, P = .03). The decrease in methylglyoxal after 6 months correlated with decrease in NDS after twelve months (r = 0.897, P = .003).

Conclusion: RYGB seems to improve oxidative, nitrosative and carbonyl stress, known to have a causal role in diabetic neuropathy. (Surg Obes Relat Dis 2015;▮:00–00.)

Keywords: Diabetes; Neuropathy; Metabolic surgery; Oxidative stress; Carbonyl stress; Gastric bypass; RYGB

Diabetic neuropathy affects 10% to 25% of patients with diabetes. One third of diabetic patients experiences painful symptoms without measurable neurologic deficits [1]. In insulin-dependent diabetic patients, the prevalence of diabetic neuropathy rises to about one third [2,3]. One of the

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major problems of diabetic neuropathy is the lack of effective treatment options for the often painful symptoms. Although it has been shown that tight glucose control improves diabetic neuropathy in type 1 diabetes mellitus (T1DM), large studies could not demonstrate a beneficial effect of tight glucose control on the symptoms and progression of diabetic neuropathy in type 2 diabetes mellitus (T2DM) [4,5].

One of the reasons for the lack of effective therapies for diabetic neuropathy is the poor understanding of its pathogenesis. Several underlying mechanisms of diabetic neuropathy have been proposed, including osmotic stress through alternative glucose metabolizing pathways (such as the sorbitol pathway); the formation of advanced glycation end products (AGE) that activate inflammatory signaling through specific receptors, resulting in activation of inflammatory pathways and the production of reactive oxygen species (ROS); the consumption of NADPH, as well as the toxic effects of free fatty acids, resulting in the activation of the protein kinase C. All of these mechanisms may contribute to oxidative stress by increasing ROS and reactive carbonyl species (RCS) formation [6,7]. Signs of nitrosative, carbonyl, and oxidative stress, as well as the formation of AGE, are the carbonylation and nitrosylation of proteins, oxidation of low density lipoprotein, and the production of methylglyoxal. All of these products have been shown to contribute to diabetic neuropathy in animal experiments [8–12]. Methylglyoxal, a product of carbonyl stress, is thus far the only substance that has been shown to directly cause neuropathic pain [13].

Besides nitrosative, carbonyl, and oxidative stress, other substances or pathways may also be involved in the pathomechanisms and symptoms of diabetic neuropathy. Neuropeptid Y (NPY) has multiple physiologic roles, but it has been shown to exacerbate peripheral hyperalgesia which makes it a possible mediator of neuropathic pain in diabetes [14,15]. Neurokinin A (NKA) is a neuropeptid of the tachykinin group and acts through the tachykinin receptors 1 and 2. The blockage of both tachykinin receptors reduces allodynia, but it appears that blockade of tachykinin receptor 2, the principle receptor of NKA, has a stronger effect on diabetic neuropathy-related pain [16].

We recently reported that Roux-Y gastric bypass (RYGB) improved symptoms of diabetic neuropathy in nonseverely obese patients with a body mass index (BMI) <35 kg/m^2 and long-standing T2DM within 3 months [17]. Hence, these patients offer a great opportunity to investigate whether postbariatric changes in diabetic neuropathy biomarkers give insight into the pathophysiological mechanisms involved in diabetic neuropathy reversal in humans. The present study was initiated to investigate whether RYGB does not only, as previously published, reduce NSS independent of glucose and HbA1c, but also affects the major inducers of active neuropathic symptoms [17]. These results may give valuable insight into the underlying mechanisms of diabetic neuropathy in humans.

Methods

Twenty patients were prospectively enrolled in this study, as previously published [17]. In brief, inclusion criteria comprised insulin-dependent T2DM, a BMI 25–35 kg/m^2, preserved insulin-secretion assessed by a glucagon-stimulated C-peptide of >1.5 ng/mL, glycated hemoglobin (HbA_{1c}) $>7\%$ (53 mmol/mol IFCC), and age 18–70 years. The study was approved by the institutional review board and was registered in the German Clinical Study Registry (DRKS00004605). Clinical data after 6 months have already been published [17].

All patients underwent a RYGB with a length of the alimentary and the biliopancreatic limb of 150 cm and 75 cm, respectively, performed by the same surgical team. Postoperatively, diabetic medication was stopped and use was reassessed depending on the patient's glucose profile and according to the diabetologist's opinion. All patients received standard, over-the-counter multivitamin and micronutrient supplements twice daily. Further dietary supplementation was substituted depending on laboratory results obtained during regular follow-up visits. A clinical assessment of neuropathy using the Neuropathy Symptom Score (NSS) and the Neuropathy Deficit Score (NDS) was conducted preoperatively, and at 6 and 12 months postoperatively, by the same experienced endocrinologist (P.P. N.) thus eliminating interrater variability [17]. Neuropathy was diagnosed when either of the scores was >3 points. For the further analysis, we chose to focus on the NDS because we believe that the NSS is more susceptible to placebo effects than the NDS which is based on more objective deficits.

At the same visits, routine laboratory tests including fasting glucose, HbA1c, and C-reactive protein (CRP) were conducted. In addition, blood was drawn and collected in EDTA-Vacutainers (BD, Heidelberg, Germany) and the plasma was collected after centrifugation. The plasma samples were stored at -80°C for further analysis. From these samples, oxidized low density lipoprotein (oxLDL, OxiSelect MDA-LDL-Quantitation), N-ε-carboxymethly-Lysine (CML, OxiSelect Competitive ELISA), and protein nitration (OxiSelect Nitrotyrosine Protein ELISA) were measured using enzyme-linked immunosorbent assays (ELISA) according to manufacturer's instructions (BioCat, Heidelberg, Germany). Neuropeptid Y (NPY) and Neurokinin A (NKA) were determined using specific enzyme immunoassays (EIA) (Sigma-Aldrich, Germany) according to the manufacturer's instructions. Samples were assessed in duplicates and diluted to fit the standard range. Plates were read with a microplate reader at 450 nm, with reference values at 540 nm (Infinte 200 Pro, Tecan, Mainz, Germany), and data were analyzed with the Magellan™ Data