Clinical PNP Studies
HighTone Therapy with HiToP®

Diabetic Polyneuropathy

Effective treatment of symptomatic diabetic polyneuropathy by high-frequency external muscle stimulation
Diabetologia (2005) 48: 824–828

Publication of the "proof of principle" study performed by Prof. S. Martin at the DDZ (German Diabetes Center, Düsseldorf).

New Possibilities for The Treatment of Type 2 Diabetes Mellitus by Means of External Electrical Muscle Stimulation
Posterpresentation at the German Diabetes Conference 2005

Posterpresentation of data gathered at above study showing a reduction of weight and an improvement in the HbA1c value after HiToP® treatment.

High frequent muscle stimulation as a treatment for painful neuropathy for type 2 diabetes improves the micro vascular endothelial cell function
Posterpresentation at the German Diabetes Conference 2006

Study performed at the University of Heidelberg showing similar results as the DDZ study and linking the positive results to an improved micro vascular endothelial cell function.

External Muscle Stimulation to Influence Adjustment of Diabetes: a New Therapy Option for Type 2 Diabetics
Posterpresentation at the German Diabetes Conference 2007

Publication: Experimental and Clinical Endocrinology & Diabetes 2008
DOI: 10.1055/s-2008-1065331 with the title: "Beneficial Effects of External Muscle Stimulation on Glycaemic Control in Patients with Type 2 Diabetes"

The study group at the University of Düsseldorf further evaluated gathered data showing improved metabolical and immunological parameters after HiToP® treatment.

Muskelstimulation mit hoch-frequenter externer Muskelstimulation mindert signifikant die Symptome der diabetischen Polyneuropathie
Presentation at the German Diabetes Conference 2008 in Munich

Publication: Diabetologie und Stoffwechsel 2008; 3 DOI: 10.1055/s-2008-1076201

Study of the WDGZ (Westdeutsches Diabetes und Gesundheitszentrum) under the responsibility of Prof. Dr. S. Martin with over 400 diabetics showing statistically significant improvements of polyneuropathy sympotoms.

High-frequency External Muscle Stimulation significantly improves Symptomatic Diabetic Polyneuropathy
Posterpresentation at the American Diabetes Association 68th Scientific Sessions
June 6 - 10, 2008, San Francisco, California

The results of the above WDGZ study with 400 diabetics were accepted as a poster presentation at the ADA.

External Electric Muscle Stimulation Improves Burning Sensations and Sleeping Disturbances in Patients with Type 2 Diabetes and Symptomatic Neuropathy
Pain Medicine Volume 10 Issue 2 (March 2009)

Clinical study at the University of Heidelberg with 100 patients. 73% of the patient showed significant improvements also regarding sleeping disturbances.

High-Frequency External Muscle Stimulation in the Relief of Symptomatic Diabetic Neuropathy
Diabetes, Stoffwechsel und Herz, Band 19, 5/2010

Publication of the study of the WDGZ (Westdeutsches Diabetes und Gesundheitszentrum) under the responsibility of Prof. Dr. S. Martin with over 400 diabetics.

Uremic Polyneuropathy

High-Tone External Muscle Stimulation in End-Stage Renal Disease: Effects on Symptomatic Diabetic and Uremic Peripheral Polyneuropathy
Presented at the 5th International Congress Of The Uremic Research And Toxiticity and 7th Baltic Meeting On Nephrology; (May 18th - 20th, 2007)

Published: Journal of Renal Nutrition, Vol 18, No 1 (January), 2008: pp 46–51

Study under the lead of Prof. A. Heidland, University of Würzburg and Prof. N. de Santo, University of Naples, showing the successful treatment of dialysis patients suffering from PNP (Polyneuropathy).

High-Tone External Muscle Stimulation in End-Stage Renal Disease: Effects on Symptomatic Diabetic and Uremic Peripheral Polyneuropathy
Posterpresentation at the ERA-EDTA Congress 10. – 13. Mai 2008 Stockholm

Abstract published: May 2008 issue of "Nephrology Dialysis Transplantation (NDT plus)", Volume 1, Supplement 2

The above mentioned study was continnued and the long term results presented.

Neuro-musculareelectro-stimulation:Outlooks for patientswith renal disease

Clinical Nephrology, Vol. 78 – Suppl. 1/2012 (S1)

Shown here is only the introduction to a 52 page publication.

High-Tone external Muscle Stimulation in patients with acute kidney injury (AKI): beneficial effects on NO metabolism, asymmetric dimethylarginine, and endothelin-1

Clinical Nephrology, Vol. 82 – No. 5/2014 (304 – 312)

Diabetologia (2005) 48: 824–828
DOI 10.1007/s00125-005-1728-0

SHORT COMMUNICATION

L. Reichstein · S. Labrenz · D. Ziegler · S. Martin

Effective treatment of symptomatic diabetic polyneuropathy by high-frequency external muscle stimulation

Received: 28 September 2004 / Accepted: 19 November 2004 / Published online: 14 April 2005

Abstract *Aims/hypothesis:* Diabetic distal symmetrical sensory polyneuropathy (DSP) affects 20–30% of diabetic patients. Transcutaneous electrical nerve stimulation (TENS) and electrical spinal cord stimulation have been proposed as physical therapies. We performed a controlled, randomised pilot trial to compare the effects of high-frequency external muscle stimulation (HF) with those of TENS in patients with symptomatic DSP. *Methods:* Patients with type 2 diabetes and DSP (n=41) were randomised to receive treatment with TENS or HF using strata for non-painful (n=20) and painful sensory symptoms (n=21). Both lower extremities were treated for 30 min daily for three consecutive days. The patients' degree of symptoms and pain were graded daily on a scale of one to ten, before, during and 2 days after treatment termination. Responders were defined by the alleviation of one or more symptoms by at least three points. *Results:* The two treatment groups were similar in terms of baseline characteristics, such as age, duration of diabetes, neurological symptoms scores and neurological disability scores. The responder rate was significantly higher (p<0.05) in the HF group (80%, 16 out of 20) than in the TENS group (33%, seven out of 21). Subgroup analysis revealed that HF was more effective than TENS in relieving the symptoms of non-painful neuropathy (HF: 100%, seven out of seven; TENS: 44%, four out of nine; p<0.05) and painful neuropathy (HF: 69%, nine out of 13; TENS: 25%, three out of 12; p<0.05). The responders did not differ in terms of the reduction in mean symptom intensity during the trial. *Conclusions/interpretation:* This pilot study shows, for the first time, that HF can ameliorate the discomfort and pain associated with DSP, and suggests that HF is more effective than TENS. External muscle stimulation offers a new therapeutic option for DSP.

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Keywords Diabetes mellitus · Distal symmetrical sensory polyneuropathy · High-frequency external muscle stimulation · Transcutaneous electrical nerve stimulation

Abbreviations DSP: distal symmetrical sensory polyneuropathy · HF: high-frequency external muscle stimulation · TENS: transcutaneous electrical nerve stimulation · TSS: total symptom score

Introduction

Diabetic distal symmetrical sensory polyneuropathy (DSP) affects approximately 20–30% of the hospital-based type 2 diabetic population and 20% of community-based samples of diabetic patients [1, 2]. Neuropathic symptoms can be categorised as being positive or negative, based on spontaneous sensory symptoms or decreased responsiveness to stimuli, respectively [3]. There are numerous types of positive sensory symptoms, and it has been suggested that they should be divided into painful and non-painful categories [3]. Because the aetiology of DSP in humans is not well understood, symptomatic treatment using analgesics, tricyclic antidepressants and anticonvulsant drugs is often the only way of alleviating the discomfort and pain reported by these patients [2]. A recently published meta-analysis provided evidence that i. v. treatment with α-lipoic acid improves positive neuropathic symptoms and neuropathic deficits in DSP [4]. Transcutaneous electrical nerve stimulation (TENS) [5, 6], percutaneous electrical nerve stimulation [7], spinal cord stimulation [8], other physical therapies [11, 12] and acupuncture [13] have also successfully been used as non-pharmacological therapies, while electrical stimulation therapy through stocking electrodes was not effective [14].

We previously noticed an alleviation of neuropathic symptoms in patients treated with high-frequency external muscle stimulation (HF). Based on this finding, the aim of this randomised pilot study was to compare the effects of HF therapy with those of established TENS therapy in patients with DSP.

Subjects and methods

Subjects Patients who had type 1 or 2 diabetes and polyneuropathy, who were aged 18–80 years, and had HbA_1c levels <11% were enrolled in the present study. Patients taking medication that may influence neuropathic symptoms (such as α-lipoic acid, tricyclic antidepressants or anticonvulsants), and patients with ulcers, amputations caused by ischaemia, or cancer diseases were excluded. Forty-one adult diabetic patients were enrolled in this study; all participants gave their written informed consent. The study protocol was approved by the Ethical Review Board of the Heinrich-Heine-University Düsseldorf.

A detailed history of diabetes and a neurological examination of the lower extremities were performed to establish eligibility (Table 1). Classical symptoms and neurological deficiencies defined DSP. Scores for neurological symptoms or neurological impairment were used to assess the severity of DSP [15]. Participants were randomly assigned to the TENS or the HF treatment group using strata for patients with non-painful (paraesthesiae, numbness) and painful neuropathic symptoms (in addition to non-painful symptoms; burning, stabbing, shooting of an electrical nature).

Physical treatment procedure Each patient was treated for 30 min on three consecutive days. In the TENS treatment group the electrodes were placed on the lower extremities as described previously [4] (Fig. 1a). Transcutaneous electrotherapy was applied with the H-Wave device (Dumo 2.4; CEFAR Medical, Lund, Sweden), a portable, rechargeable unit that generates a biphasic exponentially decaying waveform with pulse widths of 4 ms, ≤35 mA, ≤35 V and 180 Hz. Intensity was adjusted according to the patient, and ranged from 20 to 30 mA.

Table 1 Baseline characteristics of 41 adults with diabetes who participated in the study

Characteristic	TENS treatment group (n=21)	HF treatment group (n=20)
Age (years)	57.8±12.5	64.2±12.7
Diabetes duration (years)	13.0±9.6	13.7±11.5
Type of diabetes (type 1/ type 2)	3/18	4/16
Sex (women/men)	11/10	8/12
Diabetes therapy (insulin/oral hypoglycaemic drugs)	18/3	17/3
BMI (kg/m^2)	28.1±5.8	29.5±5.9
Neurological symptoms score	6.6±1.3	7.1±1.3
Total symptom score	6.6±3.2	7.0±3.6
Neurological impairment score	5.5±3.2	7.4±1.6
HbA_1c (%)	9.3±1.6	9.3±2.4
Triglycerides (mmol/l)	3.0±4.3	1.9±1.3
Cholesterol (mmol/l)	6.0±2.0	5.6±1.0
HDL (mmol/l)	1.1±0.4	1.3±0.4
LDL (mmol/l)	3.7±1.2	3.7±0.9
GOT (U/l)	24.5±13.0	25.7±13.1
GPT (U/l)	27.8±15.4	25.7±13.4
Creatinine (mmol/l)	65.5±13.3	71.6±22.1
Urea (mmol/l)	6.0±1.9	6.8±2.0

Data are means±SD
GOT Glutamate–oxalate transaminase; *GPT* glutamate–pyruvate transaminase

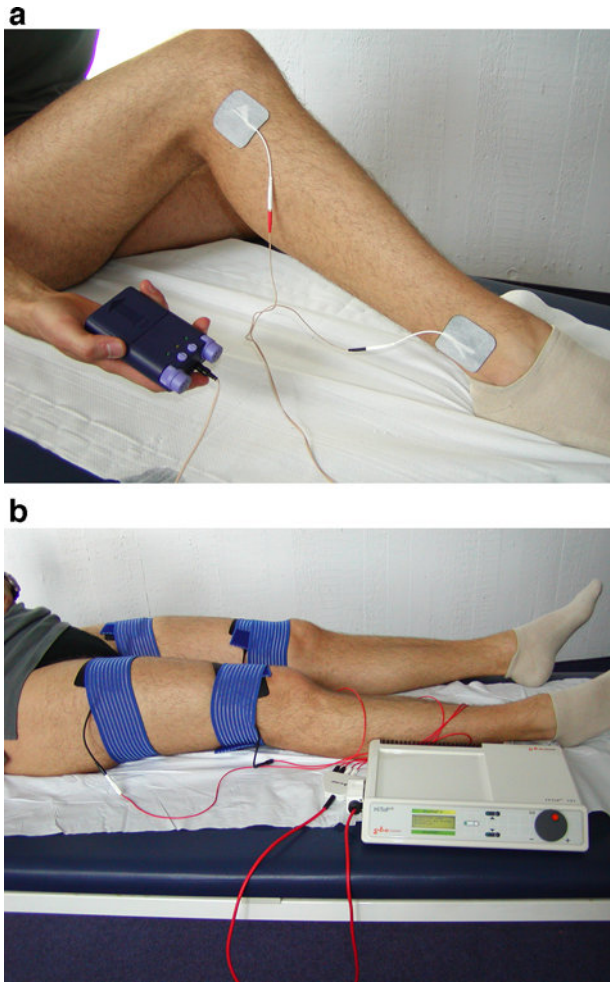


Fig. 1 Electrode placement in TENS (**a**) and HF (**b**) treatment

The electrodes for the HF therapy were placed on the femoral muscles (Fig. 1b). External muscle stimulation was performed using a non-portable 230-V power supply device (HiTop 181-H; gbo Medizintechnik, Rimbach, Germany) that generates pulse widths of ≤350 mA, ≤70 V. We used an initial frequency of 4,096 Hz, which was increased up to 32,768 Hz within 3 s; the maximum frequency was used for 3 s and then downmodulated from 32,768 to 4,096 Hz. For each patient, the intensity of the electrical stimulation was adjusted to a pleasant level that did not produce any pain or discomfortable paraesthesiae.

Grading of symptoms Patients were asked to record their baseline levels of the major symptoms—pain, numbness, numbness in painful areas, burning, paraesthesiae and dysaesthesia in the lower extremities—using separate visual linear 10-point scales, where 1=no symptom and 10=worst ever felt [16]. The questionnaires were completed by the

participants 1 day before the first treatment session, 1–2 h after the end of therapy on all three treatment days, and 2 days after therapy. An improvement of symptoms was defined as an improvement of three points or more for at least one symptom. The data were also analysed using the Total Symptom Score (TSS) [17].

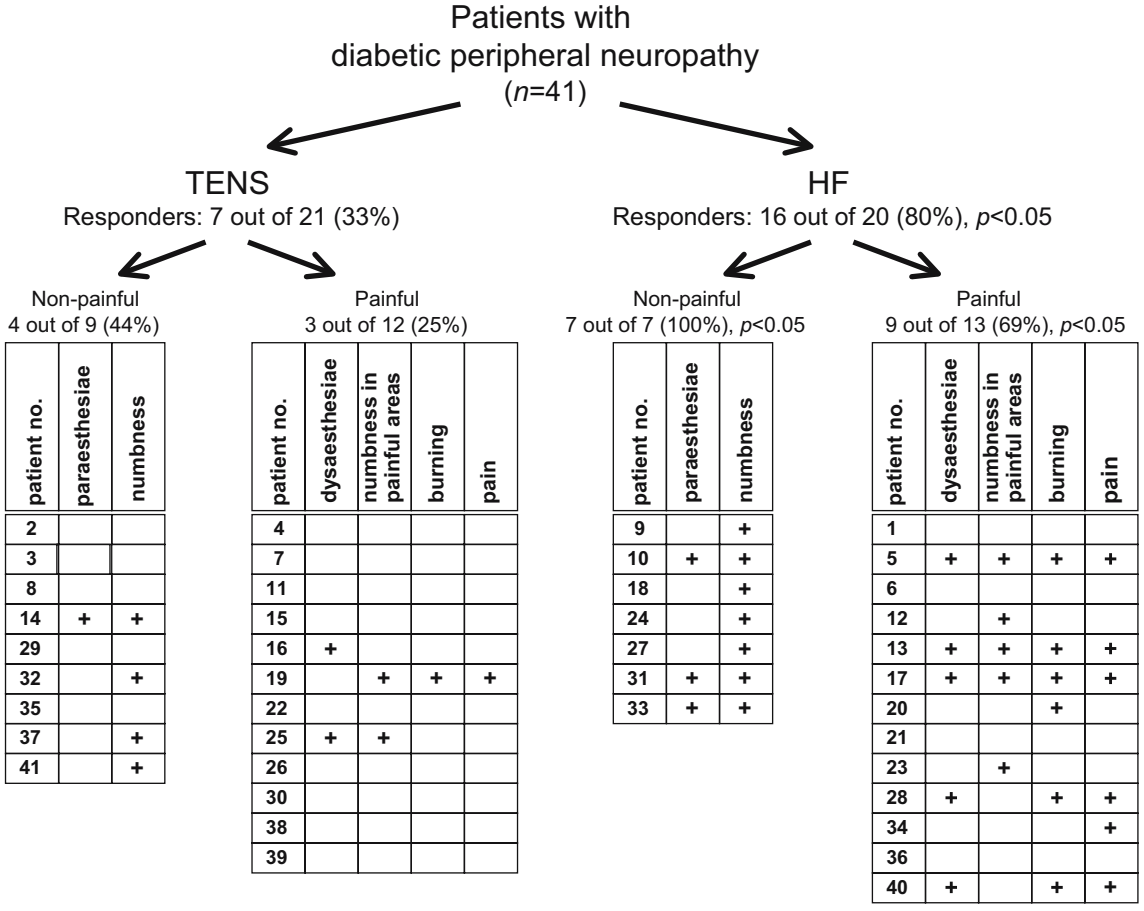
Statistical analyses All data are expressed as means±SD or as percentages. The analyses of responder data were performed using the chi square test. Changes in TSS were calculated by paired *t*-test (two-tailed). A *p* value less than 0.05 was considered statistically significant. Statistical analyses were performed using GraphPadPrism, Version 3.0 (GraphPad Software; San Diego, CA, USA).

Results

Patients were randomised to receive TENS (n=21) or HF (n=20). The demographic data, laboratory values and neurological symptoms and impairment scores at baseline were similar for the two treatment groups (Table 1). Both forms of electrical treatment were well tolerated, with no local or systemic side effects. Only one patient reported some muscular discomfort in his legs after the first HF treatment, which ceased within several hours. All 41 patients completed the study protocol.

Seven out of 21 patients (33%) in the TENS group and 16 out of 20 patients (80%) in the HF group reported an improvement of symptoms (p<0.05) (Fig. 2). Treatment with HF was more effective than TENS therapy in patients with non-painful DNP (HF: 100%, seven out of seven; TENS: 44%, four out of nine; p<0.05) and in patients with painful DNP (HF: 69%, nine out of 13; TENS: 25%, three out of 12; p<0.05) (Fig. 2). Both treatment modalities led to a significant decrease in TSS between baseline and the end of the observation period, although this reduction was more pronounced in the HF group (from 7.0±3.6 to 4.6±3.4, p<0.005) than in the TENS group (from 6.6±3.2 to 5.4±3.8, p<0.05).

Responders in both groups were analysed separately to compare the time courses for the improvement of symptoms with treatment (Fig. 3). The mean symptoms scores in responders decreased from 7.3±0.6 to 3.6±0.6 in the HF group and from 5.4±0.6 to 1.9±0.4 in the TENS group. An improvement of symptoms was reported immediately after the termination of the first treatment, with a further decrease in scores occurring during the subsequent days. A



patient no.	paraesthesiae	numbness
2		
3		
8		
14	+	+
29		
32		+
35		
37		+
41		+

patient no.	dysaesthesiae	numbness in painful areas	burning	pain
4				
7				
11				
15				
16	+			
19		+	+	+
22				
25	+	+		
26				
30				
38				
39				

patient no.	paraesthesiae	numbness
9		+
10	+	+
18		+
24		+
27		+
31	+	+
33	+	+

patient no.	dysaesthesiae	numbness in painful areas	burning	pain
1				
5	+	+	+	+
6				
12		+		
13	+	+	+	+
17	+	+	+	+
20			+	
21				
23		+		
28	+		+	+
34				+
36				
40	+		+	+

Fig. 2 Results of treatment with TENS versus HF for the complete groups and individual patients stratified into non-painful and painful groups. Responders were defined as patients who showed an improvement of three points or more for at least one symptom in the linear 10-point symptoms scales. Statistical differences were calculated using the chi square test. + An improvement of at least three points in the symptom scale

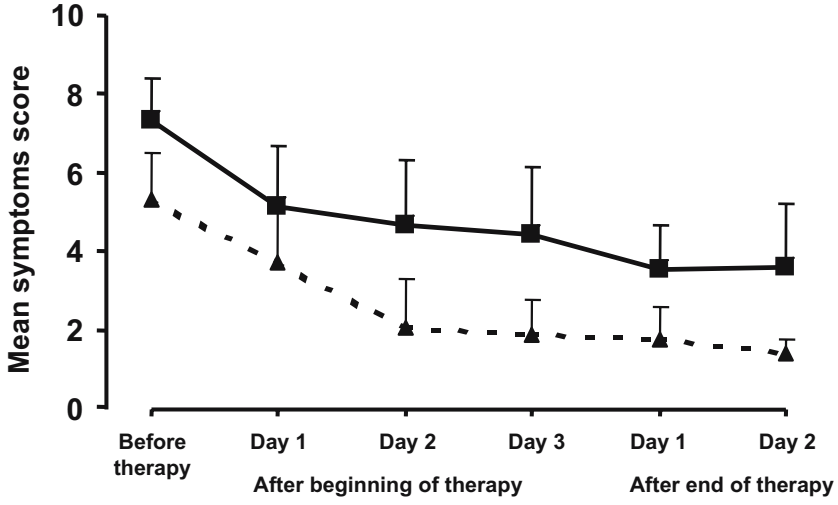


Fig. 3 Mean symptoms scores (+SD) of responders in the TENS (*black triangles*, *broken line*; *n*=7) and the HF groups (*black squares*, *solid line*; *n*=16)

reduction of symptoms was documented up to 2 days after the end of treatment. However, patients reported the recurrence of symptoms several days later, which was ameliorated by further electrotherapy (data not shown).

Discussion

In this prospective, randomised clinical pilot study we have shown, for the first time, that HF can reduce the pain and discomfort caused by DSP. This non-pharmacological form of treatment was well tolerated and might offer a new option for symptomatic relief.

Our finding that external muscle stimulation reduced neuropathic symptoms is in accordance with the clinical observation that neuropathic symptoms appear when people do not use their muscles during sleep, and disappear when they stand up or walk [18]. Treatment with TENS has previously been shown to be effective in the relief of pain and discomfort associated with DSP [5, 6]. In these studies a control group received sham treatment using placebo stimulators; however, use of this procedure does not exclude placebo effects. This problem was avoided in our study.

A special stimulation system (HiTop 181-H) was used for external muscle contraction. We applied 1-s frequencies of 4,096–32,768 Hz, introducing up to 5,000 mW into the muscles. High-frequency electrical spinal cord stimulation with implanted electrodes has previously been shown to be effective in the relief of chronic diabetic neuropathic pain [8] and several other chronic painful conditions, including back pain, phantom-limb pain, peripheral vascular disease and severe angina [19, 20]. Whereas this procedure is not free of risk and may give rise to life-threatening infection [21], the HF treatment used in our study is safe and may potentially be used for other chronic pain indications. The mechanisms that underlie the positive results associated with this therapeutic modality are not clear. It has been proposed that electrical stimulation activates the dorsal columns that inhibit the C fibres, thus interrupting/gating pain input [22]. Furthermore, short-term high-frequency electrical nerve stimulation decreases human motor cortex excitability [23]. Experiments indicate that electrical stimulation is followed by a decrease in the concentrations of the excitatory amino acids glutamate and aspartate in the dorsal horn, and that this effect is mediated by a GABAergic mechanism [24].

Microvascular changes, reduced blood flow, nerve oxygen tension [25, 26] and other vascular factors [27] contribute to the pathogenesis of diabetic neuropathy. Interestingly, electrical stimulation has been reported to improve microvascular blood flow in severe limb ischaemia [28], have effects on wound healing (indicating improved tissue circulation) [29, 30], and improve insulin resistance [31–34].

One of the limitations associated with our pilot study is its short duration, which might be responsible for the weak effect of TENS treatment. Long-term studies are needed to ascertain the cumulative effects of HF in diabetic patients. The patients' pain and discomfort was found to recur a few days after discontinuation of the therapy, implying that continuous treatment would be more beneficial. Limited experience with patients followed at our clinic suggests that treatment given weekly can reduce long-lasting neuropathic pain.

In summary, our study suggests that HF is a useful non-invasive, non-pharmacological treatment for the management of peripheral polyneuropathy in patients with diabetes.

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References

1. Harris M, Eastman R, Cowie C (1993) Symptoms of sensory neuropathy in adults with NIDDM in the U.S. population. Diabetes Care 16:1446–1452

2. Boulton AJ, Malik RA, Arezzo JC, Sosenko JM (2004) Diabetic somatic neuropathies. Diabetes Care 27:1458–1486
3. Apfel SC, Asbury AK, Bril V et al (2001) Positive neuropathic sensory symptoms as endpoints in diabetic neuropathy trials. J Neurol Sci 189:3–5
4. Ziegler D, Nowak H, Kempler P, Vargha P, Low PA (2004) Treatment of symptomatic diabetic polyneuropathy with the antioxidant alpha-lipoic acid: a meta-analysis. Diabet Med 21:114–121
5. Kumar D, Marshall HJ (1997) Diabetic peripheral neuropathy: amelioration of pain with transcutaneous electrostimulation. Diabetes Care 20:1702–1705
6. Kumar D, Alvaro MS, Julka IS, Marshall HJ (1998) Diabetic peripheral neuropathy. Effectiveness of electrotherapy and amitriptyline for symptomatic relief. Diabetes Care 21:1322–1325
7. Hamza MA, White PF, Craig WF et al (2000) Percutaneous electrical nerve stimulation: a novel analgesic therapy for diabetic neuropathic pain. Diabetes Care 23:365–370
8. Tesfaye S, Watt J, Benbow SJ et al (1996) Electrical spinal-cord stimulation for painful diabetic peripheral neuropathy. Lancet 348:1698–1701
9. Weintraub MI, Wolfe GI, Barohn RA et al (2003) Static magnetic field therapy for symptomatic diabetic neuropathy: a randomized, double-blind, placebo-controlled trial. Arch Phys Med Rehabil 84:736–746
10. Khaodhiar L, Niemi JB, Earnest R et al (2003) Enhancing sensation in diabetic neuropathic foot with mechanical noise. Diabetes Care 26:3280–3283
11. Leonard DR, Farooqi MH, Myers S (2004) Restoration of sensation, reduced pain, and improved balance in subjects with diabetic peripheral neuropathy: a double-blind, randomized, placebo-controlled study with monochromatic near-infrared treatment. Diabetes Care 27:168–172
12. Zinman LH, Ngo M, Ng ET et al (2004) Low-intensity laser therapy for painful symptoms of diabetic sensorimotor polyneuropathy: a controlled trial. Diabetes Care 27:921–924
13. Ewins DL, Vileikyte L, Borg-Costanzi J, Carrington A, Boulton AJ (1995) A novel treatment for painful neuropathy. In: Hotta N, Greene DA, Ward JD (eds) Diabetic neuropathy, new concepts and insights. Excerpta Medica, Amsterdam, pp 405–408
14. Oyibo SO, Breislin K, Boulton AJ (2004) Electrical stimulation therapy through stocking electrodes for painful diabetic neuropathy: a double blind, controlled crossover study. Diabet Med 21:940–944
15. Young MJ, Boulton AJ, MacLeod AF, Williams DR, Sonksen PH (1993) A multicentre study of the prevalence of diabetic peripheral neuropathy in the United Kingdom hospital clinic population. Diabetologia 36:150–154
16. Galer BS, Jensen MP (1997) Development and preliminary validation of a pain measure specific to neuropathic pain: the neuropathic pain scale. Neurology 48:332–338
17. Ziegler D, Hanefeld M, Ruhnau KJ et al (1995) Treatment of symptomatic diabetic peripheral neuropathy with the anti-oxidant alpha-lipoic acid: a 3-week multicentre randomized controlled trial (ALADIN study). Diabetologia 38:1425–1433
18. Watkins PJ (1984) Pain and diabetic neuropathy. Br Med J (Clin Res Ed) 288:168–169
19. North RB, Kidd DH, Zahurak M, James CS, Long DM (1993) Spinal cord stimulation for chronic, intractable pain: experience over two decades. Neurosurgery 32:384–394
20. TenVaarwerk IA, Jessurun GA, DeJongste MJ et al (1999) Clinical outcome of patients treated with spinal cord stimulation for therapeutically refractory angina pectoris. The Working Group on Neurocardiology. Heart 82:82–88
21. Torrens JK, Stanley PJ, Ragunathan PL, Bush DJ (1997) Risk of infection with electrical spinal-cord stimulation. Lancet 349:729
22. Watkins ES, Koeze TH (1993) Spinal cord stimulation and pain relief. BMJ 307:462
23. Mima T, Oga T, Rothwell J et al (2004) Short-term high-frequency transcutaneous electrical nerve stimulation decreases human motor cortex excitability. Neurosci Lett 355:85–88
24. Lundeberg T, Kjartansson J, Samuelsson U (1988) Effect of electrical nerve stimulation on healing of ischaemic skin flaps. Lancet 2:712–714
25. Tesfaye S, Harris ND, Wilson RM, Ward JD (1992) Exercise-induced conduction velocity increment: a marker of impaired peripheral nerve blood flow in diabetic neuropathy. Diabetologia 35:155–159
26. Tesfaye S, Harris N, Jakubowski JJ et al (1993) Impaired blood flow and arterio–venous shunting in human diabetic neuropathy: a novel technique of nerve photography and fluorescein angiography. Diabetologia 36:1266–1274
27. Stevens MJ, Feldman EL, Greene DA (1995) The aetiology of diabetic neuropathy: the combined roles of metabolic and vascular defects. Diabet Med 12:566–579
28. Jacobs MJ, Jorning PJ, Joshi SR et al (1988) Epidural spinal cord electrical stimulation improves microvascular blood flow in severe limb ischemia. Ann Surg 207:179–183
29. Baker LL, Chambers R, DeMuth SK, Villar F (1997) Effects of electrical stimulation on wound healing in patients with diabetic ulcers. Diabetes Care 20:405–412
30. Lundberg TCM, Ericksson SV, Malm M (1992) Electrical nerve stimulation improves healing of diabetic ulcers. Ann Plast Surg 29:328–331
31. Goodyear LJ, King PA, Hirshman MF et al (1990) Contractile activity increases plasma membrane glucose transporters in absence of insulin. Am J Physiol 258:E667–E672
32. Lund S, Holman GD, Schmitz O, Pedersen O (1995) Contraction stimulates translocation of glucose transporter GLUT4 in skeletal muscle through a mechanism distinct from that of insulin. Proc Natl Acad Sci U S A 92:5817–5821
33. Chilibeck PD, Bell G, Jeon J et al (1999) Functional electrical stimulation exercise increases GLUT-1 and GLUT-4 in paralyzed skeletal muscle. Metabolism 48:1409–1413
34. Hamada T, Hayashi T, Kimura T, Nakao K, Moritani T (2004) Electrical stimulation of human lower extremities enhances energy consumption, carbohydrate oxidation, and whole body glucose uptake. J Appl Physiol 96:911–916

New Possibilities for The Treatment of Type 2 Diabetes Mellitus by Means of External Electrical Muscle Stimulation

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German Diabetes Center

Background and Objectives

Lately it was shown in a study, that by external electrical muscle stimulation (EMS) the sensitivity to insulin is being improved. By means of a functional EMS obviously the distribution of glucose-transport-protein GLUT-1 and GLUT-4 is being increased. Aim of the study was to examine whether a 6 week „treatment" with EMS of patients with type 2 diabetes (T2DM) will lead to the reduction of body weight, BMI and HbA_{1c}.

figure 1

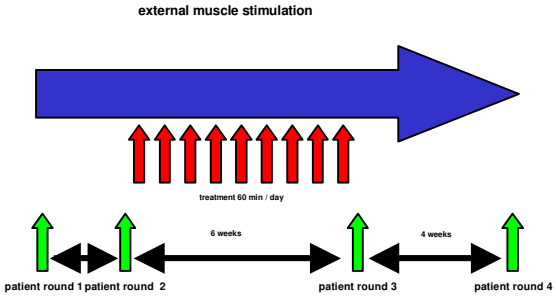
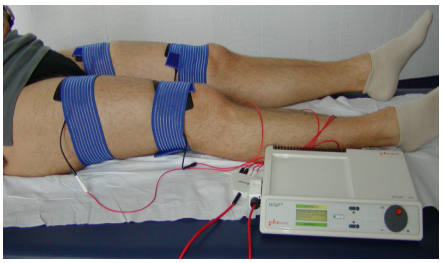


figure 2



Methods

T2DM patients, who were only treated with a diet and/or oral anti diabetics were included in this 12 week study. After an introductory phase of 2 weeks with the use of an EMS unit, the HiToP® 191 unit (gbo Medizintechnik AG, Rimbach/Germany) was given at the patients' disposal. On average, the test persons used the unit daily during the following 6 weeks. Alternately, the electrodes were placed in the area of the musculature of thighs and the shank. Each period of application and intensity was recorded by the units. After this 6 week period of treatment the units were given back. The course of the above mentioned parameters was documented further for the following 4 weeks without EMS.

figure 3

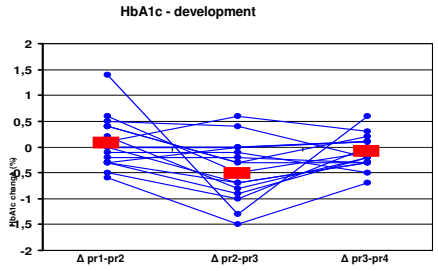
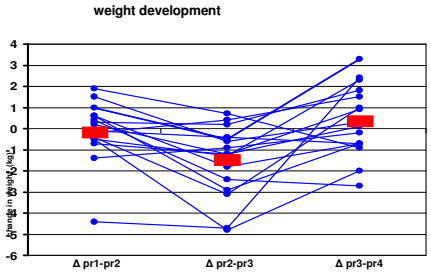


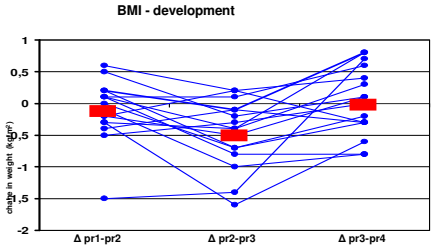
figure 4



Results

A total of 16 patients with T2DM (ages 58 ±11; 69% male; BMI 34 ±5 kg/m²; HbA_{1c} 7.5 ±1%; period of diabetes 8 ± 6 years) were examined. After a 6 week treatment a significant reduction in body weight (107.2 vs. 105.5 kg; $p<0.05$) as well as a reduction in the HbA_{1c} (7.5 vs. 7.1%; $p=0.08$) was observed. The average weight reduction was at 1.4 kg, the BMI was reduced by 0.6 kg/m² and the HbA_{1c} by 0.6%.

figure 5



Conclusions

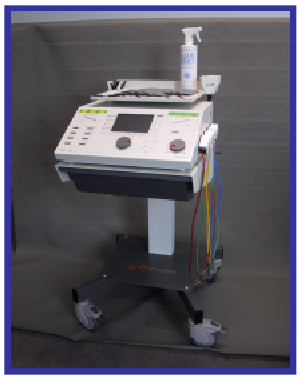
In the framework of this study, for the first time a positive effect on EMS on clinically relevant end points of patients with T2DM could be demonstrated. Further controlled studies will show, whether the use of EMS will represent a new possibility for the (additive) treatment of T2DM.

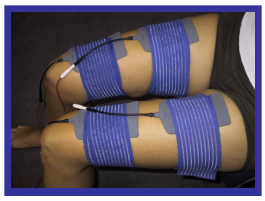
UniversitätsKlinikum Heidelberg

High frequent muscle stimulation as a treatment for painful neuropathy for type 2 diabetes improves the micro vascular endothelial cell function

Per M. Humpert, Gottfried Rudofsky, Michael Morcos, Angelika Bierhaus, Peter P. Nawroth

Department for Internal Medicine and Clinical Chemistry





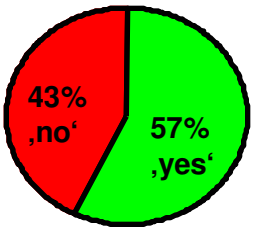
Patientcharacteristics (n=27)

Age	66,1 (±6,9)
Sex (f/m)	11/17
Duration of Diabetes	10 (±8)
HbA1c	6,7 (± 0,8)
BMI	31,3 (±5,3)
NDS	5,4 (±2,6)
NSS	7,5 (±1,2)

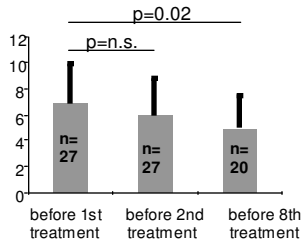
Methodology: High tone therapy was applied to the patients twice a week for 60 minutes and for a total of eight weeks. With the aid of visual analog scales (1= no pain, 10 = very strong pain) the participants' extent of the symptoms (prickle, burning, pain, numbness) before and after high tone therapy was evaluated.

Technically speaking the classical electrotherapy is an amplitude modulation: The frequency of the applied current remains constant while the current intensity is modulated, i.e. varies over time. In Low Frequency region modulation frequencies of 0 to 200 Hz are being used while the middle frequency range uses carrier frequencies of typicall 4.000 Hz. In High Tone Therapy both, amplitude (intensity of the current) and frequency, are modulated at the same time. According to the individual threhold curve for current sensation the higher the frequency the higher is the energy that can be introduced to the body. Therefor it is a „Simultaneous Frequency and Amplitude Modulation". The HiToP High Tone Therapy unit uses the frequency range from 4.000 to 32.000Hz.

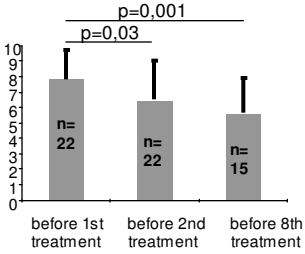
1. Subjective improvement of the pathology



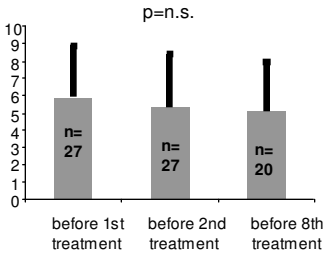
2. Pain, Foot Complete cohort



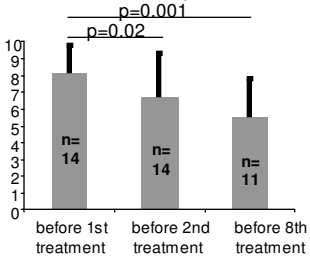
3. Pain, Foot Pain scale > 5



4. Prickle, Foot Complete cohort

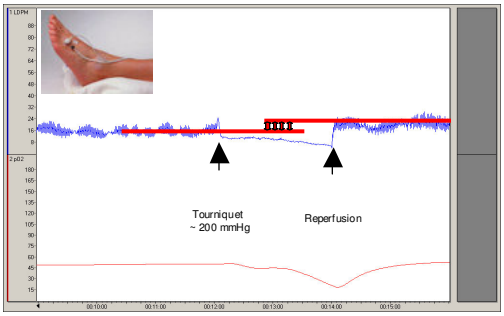


5. Prickle, Foot Scale > 5

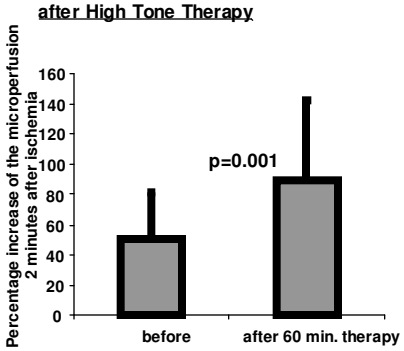


6. Laserdoppler – Measurement of the Microcirculation on the back of the foot

With a Laser Doppler Unit the relative increase of the capillary blood flow in the skin on the back of the foot after a 2 minute vessel occlusion was measured before and after a 1 hour High Tone treatment.



7. Improved micro vascular endothelial cell function after High Tone Therapy



8. Conclusions:

- High Tone therapy reduces pain sensation and prickle paraesthesia for patients with diabetes type 2
- High Tone therapy improves the micro vascular endothelial cell function
- The effect of the treatment on symptoms and micro circulation have to be correlated in larger cohorts in order to analyze the patho-physiological connection
- Limitations of the study: no placebo intervention possible

This study was supported by gbo Medizntechnik AG

External Muscle Stimulation to Influence Adjustment of Diabetes: a New Therapy Option for Type 2 Diabetics

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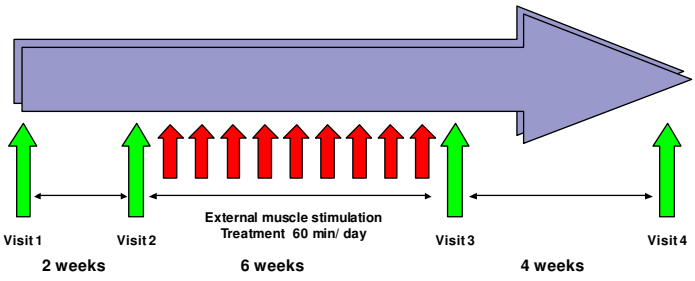
BACKGROUND AND AIMS

By the help of physical training insulin sensitivity and the adjustment of metabolism with diabetics can be improved. By means of other studies it was additionally shown that physical training will finally lead to a reduction of immunity parameters like Interleucin-6 (IL-6). It was the aim of this study to find out whether a six week treatment with a high frequent muscle stimulation (EMS) with patients suffering from Type 2 diabetes (T2D), will additionally improve metabolical and immunological parameters.

METHODOLOGY

During this 12-week study 16 patients with T2D and oral anti diabetical medication were included. After a two week Run-In phase without treatment, patients were given an EMS-device (HiToP 191, gbo Medizintechnik AG, Rimbach/Germany) for a daily treatment within the area of thigh and shank for the following 6 weeks. In the beginning as well as in the end of the treatment phase, blood was taken from the subjects and examined for metabolical and immunological parameters (TNF-α, IL-6, IL-18, Adiponektin) by means of highly intensive ELISA.

Study design



picture 1 study design

age (years)	57 ± 11
gender (f/m))	4/12
weight (kg)	107 ± 15
BMI (kg/m²)	34.5 ± 5.2
waisst (cm)	116 ± 16
circumference of waist (cm)	116 ± 9
HbA1c (%)	7.4 ± 1.1
blood sugar fasting (mg/dl)	159 ± 37
period with diabetes (years)	6.8 ± 4.8

Fig. 1 clinical charasteristics of the study population

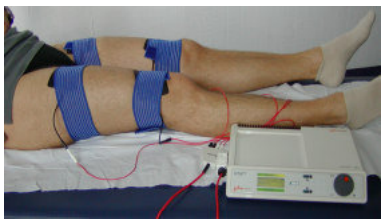


Fig. 2 EMS-device in operation

CONCLUSION

A short term treatment with a high frequent external muscle stimulation (EMS) has a positive influence on the body weight and can improve the adjustment of the blood sugar. Possibly, also the subclinical inflammation with patients with T2D is being influenced. These results point out that EMS is an additional option for the treatment of type 2 diabetics.

RESULTS

Improvement of the Adjustment of the Metabolism

During the treatment phase, a signifcant improvement of the weight, BMI and the HbA1c value in comparison to the run-in phase (**Fig. 2**) was noticed whereas the fasting blood sugar level, c-pepid level and the circumference of the waist remained largely unchanged.

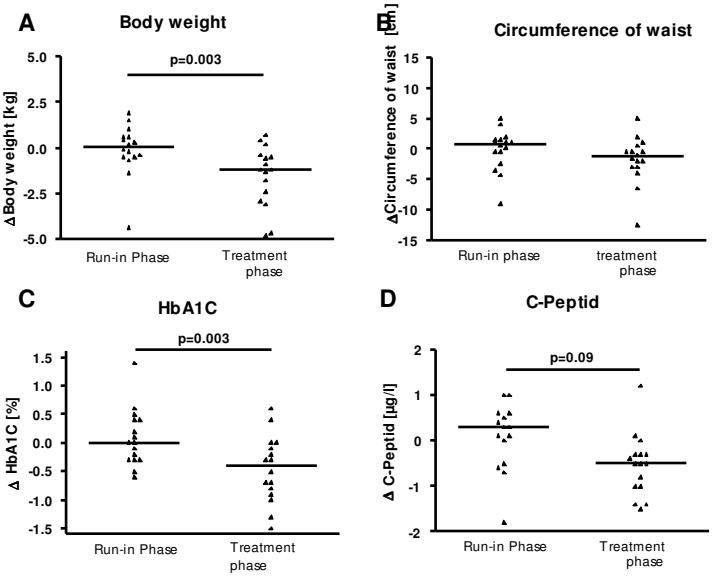


Fig. 2 Shown are the changes in body weight (A), circumferene of waist (B), HbA1c (C) and the C-Peptid level during the Run-in and the treatment phase. Each point represents an individuum, the line marks the median. The analysis was carried out with the Wilcoxon matched pairs Test.

Influence of an EMS-treatment on systemic immun parameters

With 12 of the 16 patients (75%) the HbA1c decreased during the treatment phase. Within this sub group a tendence of changes in the systemical immun parameters IL-6 und IL-18 was observed (**Fig. 3**).

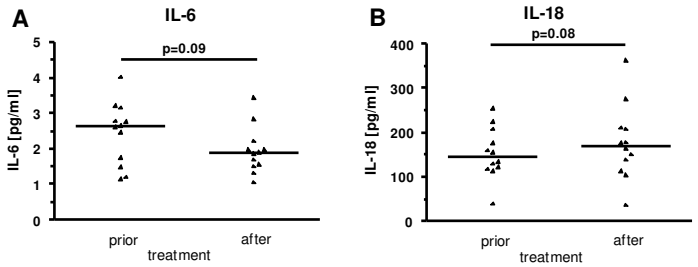


Fig. 3 Shown are the serum levels of the Zytokine IL-6 (A) and IL-18 (B) prior and after treatment with EMS within the sub group of the HbA1c-responders. Each point represents an individuum, the line marks the median. The analysis was carried out with the Wilcoxon matched pairs test.

	Treatment		
	prior	after	**p-Wert**
IL-6 (pg/ml)	2.4 ± 0.9	2.0 ± 0.7	**0.09**
IL-18 (pg/ml)	153 ± 57	177 ± 84	**0.08**
MCP-1 (pg/ml)	174 ± 66	182 ± 56	0.57
RANTES (ng/ml)	28.6 ± 16.4	33.0 ± 25.0	0.73
IP-10 (pg/ml)	334 ± 202	342 ± 158	0.79
Adiponektin (ng/ml)	6.124 ± 5.375	6.513 ± 6.418	0.68
hsCRP (mg/l)	3.2 ± 2.2	2.4 ± 2.2	0.15
Leukozyt (/µl)	6.419 ±1.249	6.375 ±1.599	0.79

Tab. 2 Serum level of the immun parameters prior and after the treatment with EMS in the group of HbA1A c-responders. The analysis was carried out with the Wilcoxon matched pairs test.

Beneficial Effects of External Muscle Stimulation on Glycaemic Control in Patients with Type 2 Diabetes

Authors

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Key words
- external muscle stimulation
- metabolic control
- type 2 diabetes
- cytokines

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Bibliography
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Abstract

Physical activity improves insulin sensitivity and metabolic control in patients with type 2 diabetes. Moreover, regular exercise can reduce systemic levels of immune markers associated with diabetes development. As patients with physical impairments are not able to exercise sufficiently, the aim of this study was to investigate whether high-frequency external muscle stimulation (hfEMS) improves metabolic and immunologic parameters in patients with type 2 diabetes and might therefore serve as complementary lifestyle therapy. Sixteen patients (12 men/4 women, age 57 ± 11 years (mean ± SD); BMI 34.5 ± 5.2 kg/m^2; HbA1c 7.4 ± 1.1 %) on oral antihyperglycaemic therapy were enrolled in this study. After a run-in phase of 2 weeks, every patient received an hfEMS device (HITOP 191, gbo-Medizintechnik AG, Rimbach/Germany) for daily treatment of femoral musculature for 6 weeks. Thereafter, patients were followed up for additional 4 weeks without hfEMS treatment. At each visit, clinical parameters were assessed and blood samples were drawn for metabolic and immunologic parameters. Immune markers (cytokines, chemokines, adipokines and acute-phase proteins) representative for the different arms of the immune system were analysed. hfEMS treatment resulted in significant reductions of body weight (−1.2 kg [−2.7 kg; −0.5 kg]; $p < 0.05$; median [25th percentile; 75th percentile]), BMI (−0.4 kg/m^2 [−0.8 kg/m^2; −0.1 kg/m^2]; $p < 0.05$) and HbA1c (−0.4 % [−0.9 %; −0.1 %]; $p < 0.05$) which were sustained during the follow-up period. Systemic levels of IL-18 tended to be increased after hfEMS treatment (171 vs. 149 pg/ml; $p = 0.06$), while all other immune markers remained virtually unchanged. Treatment with hfEMS in this first proof-of-principle study has beneficial effects on body weight and improves glycaemic control in patients with type 2 diabetes, which may be associated with changes in subclinical inflammation. Taken together, hfEMS might represent an additional treatment option for patients with type 2 diabetes not being able to exercise.

Introduction

Lack of physical activity and increased caloric intake are the main risk factors for the development of obesity and the induction of insulin resistance in peripheral tissues, especially in muscle tissue. Nowadays, insulin resistance in combination with impaired insulin secretion are thought to be the major causes of type 2 diabetes. But in contrast to insulin secretion, peripheral insulin resistance can be modified by lifestyle interventions consisting of dietary changes and increased physical activity (Houmard et al., 2004; Ross et al., 2000). Thus, lifestyle modifications are included as first-line therapy in newly diagnosed patients with type 2 diabetes into current guidelines (Standards of medical care in diabetes, 2007). Even at later stages of the disease exercise has a major impact on glucose control (Walker et al., 1999; Lindstrom et al., 2003).

But as observed in general practice, the motivation of patients to increase their physical activity is relatively low, and strong efforts have to be made to maintain high exercise levels in patients over a longer time period (Kirk et al., 2003; Krug et al., 1991). Thus, alternative treatment options with comparable effects as voluntary physical activity are of great therapeutic relevance. High-frequency external muscle stimulation (hfEMS) is the percutaneous electrical stimulation of skeletal muscles using frequencies higher than 40 Hz to produce a muscle contraction (Requena et al.,

2005). The method is widely used in orthopaedic rehabilitation, sports medicine and the treatment of peripheral nervous system lesions. It has been shown to be effective in the prevention of muscle atrophy following denervation, in the regain of muscle strength during rehabilitation to shorten rehabilitation time after sport injuries (Hainaut and Duchateau, 1992) and the treatment of symptomatic diabetic polyneuropathy (Reichstein et al., 2005). Hamada et al. could show that short-term EMS acutely increases whole body insulin sensitivity to higher levels than voluntary exercise (Hamada et al., 2004). Taken together, high-frequency EMS might be an additional therapeutic option to include an effective lifestyle-like treatment into the therapeutic regimen of patients with diabetic complications like severe heart disease or diabetic foot syndrome, who are not able to be physically active.

In addition, physical exercise has also an impact on components of the immune system, i.e. cytokines, chemokines and acute-phase proteins (APP), that are associated with type 2 diabetes and prediabetic states (Herder et al., 2005; Muller et al., 2002) and play a crucial role in the development of type 2 diabetes (Kolb and Mandrup-Poulsen, 2005). Acute physical activity is associated with a release of cytokines from the muscle (extensively reviewed in Petersen and Pedersen, 2005; Huang et al., 2007) that may exert anti-inflammatory effects, and indeed regular exercise as well as EMS are associated with reduced production of proinflammatory cytokines (Smith et al., 1999; Karavidas et al., 2006). Thus, physical activity and EMS represent also a causal therapeutic strategy in type 2 diabetes.

Therefore, the aim of our study was to investigate whether the regular use of high-frequency EMS exerts beneficial effects on body weight, glucose control and immune activation and might therefore serve as a novel treatment option in patients with type 2 diabetes.

Materials and Methods

Study population

Patients with type 2 diabetes were eligible for the study if they were aged 18–70 years, had a BMI >27 kg/m^2 and were free of insulin treatment, severe diabetes-related complications or cardiac pacemaker. Sixteen patients (12 men/4 women) were enrolled in this study and gave their written informed consent. The study was performed according to the Declaration of Helsinki and was approved by the local ethics committee at Heinrich-Heine University Duesseldorf.

Study design

After a run-in phase of 2 weeks, every patient received an hfEMS device (HITOP 191, gbo-Medizintechnik AG, Rimbach/Germany) for treatment of the femoral musculature for at least one hour per day over 6 weeks. The hfEMS device generated pulse widths of ≤350 mA, ≤70 V with an initial frequency of 4,096 Hz that were increased over 3 s to 32,768 Hz, held at maximum for 3 s and then downmodulated to the initial frequency. Each patient adjusted the intensity of the electric stimulation to a personally pleasant level that did not produce any pain or discomfort. Duration of daily use was documented in an automatic log file of the hfEMS device to assess patients' compliance. After the treatment phase, patients were followed up for additional 4 weeks without using the hfEMS device to assess the long-term effects. Study site visits took place before and after run-in, treatment and follow-up period (**Fig. 1**). At baseline visit, diabetes history and current medication were assessed and at each visit, anthropometric parameters were documented and blood samples were drawn for the measurement of metabolic and immunologic parameters.

Laboratory and immunological measurements

Blood glucose, HbA1c and serum C-peptide were measured in the central laboratory of the German Diabetes Center using standardised methods. HOMA-IR was calculated using the computer algorithm proposed by Levy et al. based on fasting blood glucose and C-peptide levels (Levy et al., 1998). One additional serum sample was drawn, centrifuged and stored at −80 °C for the analysis of cytokines, chemokines, adipokines and acute-phase proteins. The cytokines tumour necrosis factor (TNF)-α, tumour growth factor (TGF)-β, interleukin (IL)-6, IL-10, the chemokine RANTES and the adipokine adiponectin were analysed using high-sensitive ELISA (R&D Systems, Wiesbaden, Germany). High-sensitive C-reactive protein (hsCRP) was analysed using high-sensitivity latex enhanced nephelometric assay on a BN II analyser (Dade Behring, Marburg, Germany), serum amyloid A (SAA) was also analysed by immunonephelometry (Behring). Serum levels of IL-1α, IL-8, IL-17, IL-18, MCP-1, MIP-1α, MIF and IP-10 were measured by bead-based multiplex technology using a Luminex 100 analyser (Luminex Corporation, Austin, TX, USA) based on a previously published protocol (Herder et al., 2007). Fluorescent xMAP COOH microspheres were purchased from Luminex Corporation. Recombinant protein for IL-8 was obtained from Strathmann Biotec GmbH (Hamburg, Germany), recombinant IL-18 protein and anti-IL-18 antibody pair was purchased from MBL (Nagoya, Japan). All other recombinant proteins and antibody pairs were purchased from R&D Systems. The intra- and interassay coefficients of variation (CV) of quality control test sera were <10% and <20%, respectively.

Statistical analyses

Data are expressed as mean ± SD or median (25th percentile, 75th percentile) as indicated within the figure/table legend. Data were analysed using GraphPad Prism Version 4.03 (GraphPad Software, San Diego, CA, USA). For comparison of medians of paired observations, Wilcoxon signed rank test or Friedman test and an appropriate post test were used, p values ≤0.05 were considered significant.

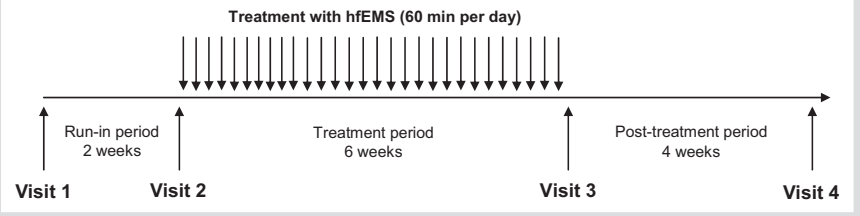

Fig. 1 Study design. After a run-in period of 2 weeks, patients used an hfEMS device daily for 60 min over 6 weeks (treatment period). Thereafter, patients remained without hfEMS treatment during the 4-week post-treatment period.

Results

Reduction of BMI and HbA1c after hfEMS treatment

Study participants had a mean age of 57 ± 11 years and were obese (BMI 34.5 ± 5.2 kg/m^2; waist circumference 116 ± 6 cm). All patients had type 2 diabetes with HbA1c levels of 7.4 ± 1.1 % and an average diabetes duration of 7 ± 5 years (**Table 1**). Oral antidiabetic medication remained unchanged or was reduced due to low blood glucose levels throughout the study, the stability of glycaemic control prior to treatment was verified during the two week run-in phase.

To evaluate the impact of hfEMS therapy on anthropometric and metabolic parameters, the change during the run-in period was calculated and compared with the change during the treatment period. To address whether hfEMS therapy has longer-lasting effects beyond the end of EMS treatment, the difference between the beginning of treatment (visit 2) and the end of study (visit 4) was also analysed. Treatment with high-frequency EMS induced significant reductions in weight (−1.2 kg [−2.7 kg; −0.5 kg]; p < 0.05), BMI (−0.4 kg/m^2 [−0.8 kg/m^2; −0.1 kg/m^2]; p < 0.05) and HbA1c (−0.4 % [−0.9 %; −0.1 %]; p < 0.05) (**Fig. 2**). This reduction was paralleled by a decrease of serum C-peptide levels (−0.5 μg/l; p < 0.05) and HOMA-IR (−0.3; p < 0.05), indicating a reduction of insulin resistance by hfEMS therapy. The observed reduction of weight, BMI, HbA1c and serum C-peptide remained almost stable after the end of hfEMS therapy in the post-treatment period. However, significance was only reached for the reduction in HbA1c (−0.6 %; p < 0.05). Waist circumference was slightly reduced after the hfEMS treatment (−1.3 cm), but this change was not statistically significant, and no changes were observed for fasting blood glucose (data not shown). Taken together, hfEMS therapy reduced body weight and improved metabolic control with possible long-term effects.

Table 1 Baseline characteristics of the study population. Anthropometric, clinical and metabolic baseline variables are given as mean ± SD

age (years)	57.3 ± 11.0
sex (f/m)	4/12
weight (kg)	107.3 ± 15.1
BMI (kg/m^2)	34.5 ± 5.2
waist circumference (cm)	116.0 ± 15.6
hip circumference (cm)	116.2 ± 8.6
HbA1c (%)	7.4 ± 1.1
fasting blood glucose (mg/dl)	159 ± 37
C-peptide (μg/l)	2.8 ± 1.2
diabetes duration (years)	6.8 ± 4.8

Impact of hfEMS treatment on immune markers

To investigate the impact of hfEMS treatment on subclinical inflammation, we analysed the levels of circulating immune markers before and after treatment in all study participants (n = 16) (**Table 2**). As hfEMS treatment has the most pronounced impact on HbA1c levels, an additional exploratory analysis was performed in therapy responders, who were defined as those with a reduction in HbA1c levels of at least 0.1 % after treatment. According to this approach, 12 of 16 patients (75 %) were classified as responders. After hfEMS treatment, systemic levels of the cytokine IL-18 tended to be higher (171 vs. 149 pg/ml; p = 0.06), while levels of other immune parameters and adipokines remained unchanged. This tendency for IL-18 was virtually identical in the responder subgroup (171 vs. 145 pg/ml; p = 0.08). Moreover, a slight reduction of IL-6 levels were observed (1.9 vs. 2.6 pg/ml; p = 0.09). Restricting

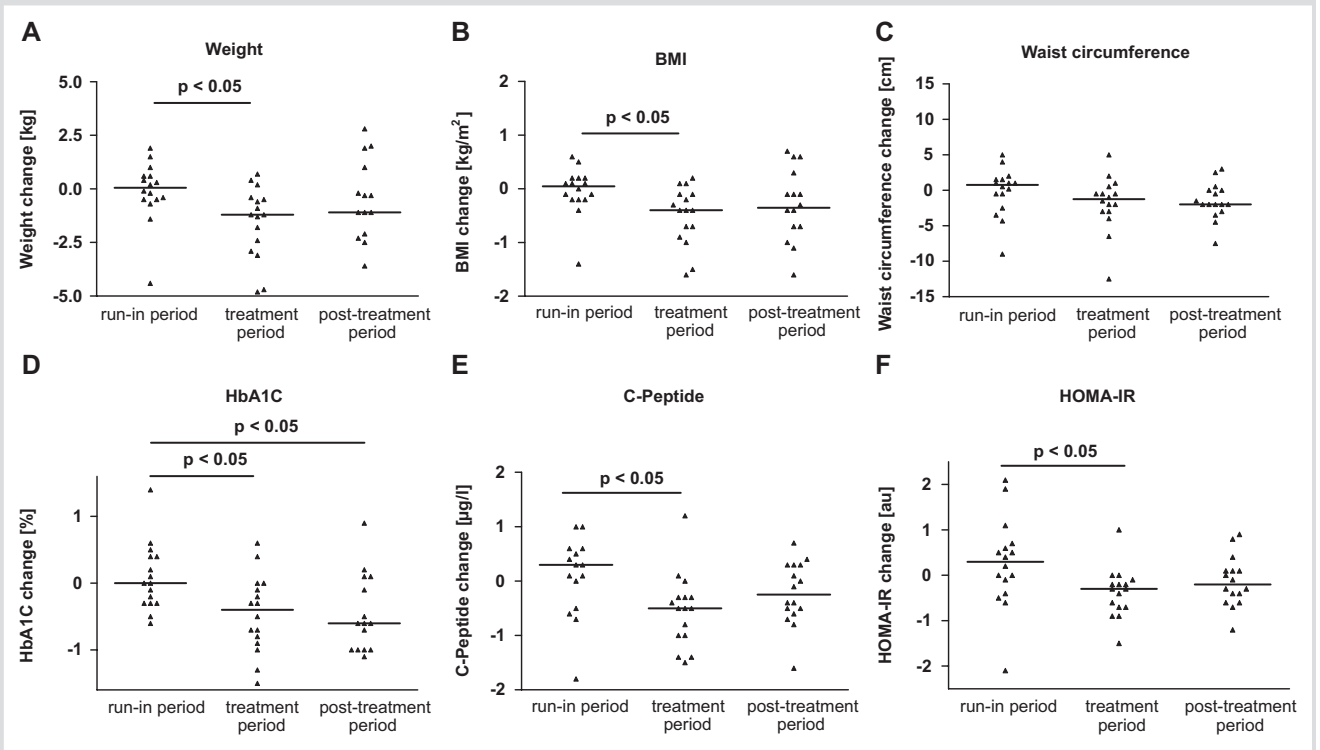

Fig. 2 Differences in anthropometric and metabolic variables before and after hfEMS therapy. The difference of weight (**A**), BMI (**B**), waist circumference (**C**), HbA1c (**D**), serum C-peptide levels (**E**) and HOMA-IR (**F**) were calculated before and after run-in (visit 2–visit 1), treatment period (visit 3–visit 2) and after post-treatment period (visit 4–visit 2). Each symbol represents one individual, the bar indicates the median. Data were analysed using Friedman test for paired observations and Dunn's post test. p-values for comparisons between different time-points are indicated by horizontal lines.

Table 2 Levels of systemic immune parameters before and after hfEMS treatment. The serum levels of immune markers are given as median (25th; 75th percentiles) before (visit 2) and directly after the 6 week hfEMS treatment period (visit 3) in all study participants (n = 16) and in the subgroup of HbA1c-responders (n = 12). Data were analysed using Wilcoxon signed rank test and p-values for the comparison between the two time-points are given

	All study participants (n = 16)			HbA1c responder (n = 12)		
Parameter	**before treatment**	**after treatment**	**p-value**	**before treatment**	**after treatment**	**p-value**
cytokines						
IL-1α [pg/ml]	2.5 (1.5; 3.5)	1.9 (1.5; 5.2)	0.28	2.9 (2.1; 7.9)	3.6 (1.8; 11.7)	0.13
IL-6 [pg/ml]	2.6 (1.4; 3.2)	1.9 (1.4; 2.5)	0.58	**2.6 (1.6; 3.0)**	**1.9 (1.5; 2.1)**	**0.09**
IL-17 [pg/ml]	2.7 (1.8; 3.9)	3.2 (1.2; 6.3)	0.17	3.1 (2.4; 6.1)	4.2 (1.8; 9.2)	0.21
IL-18 [pg/ml]	**149 (121; 191)**	**171 (120; 210)**	**0.06**	**145 (121; 192)**	**171 (127; 210)**	**0.08**
TNF-α [pg/ml]	1.9 (1.3; 2.3)	1.9 (1.1; 2.4)	0.26	2.0 (1.3; 2.5)	2.0 (1.1; 2.5)	0.30
TGF-β [ng/ml]	35.5 (29.3; 41.0)	36.9 (30.6; 43.0)	0.73	35.5 (30.3; 42.6)	36.9 (32.7; 42.3)	0.85
chemokines/adipokines						
IL-8 [pg/ml]	4.5 (1.9; 5.5)	3.8 (1.9; 7.4)	0.45	4.4 (1.9; 5.0)	4.2 (1.8; 7.4)	0.15
IP-10 [pg/ml]	283 (217; 314)	320 (237; 355)	0.54	305 (215; 348)	320 (237; 343)	0.79
MCP-1 [pg/ml]	164 (137; 194)	166 (128; 207)	0.69	166 (118; 219)	181 (132; 208)	0.57
MIF [ng/ml]	10.4 (9.2; 11.6)	9.5 (8.1; 11.7)	0.42	10.4 (9.3; 11.5)	9.2 (8.0; 11.2)	0.27
MIP-1α [pg/ml]	22.4 (12.8; 35.4)	27.3 (17.6; 45.7)	0.33	28.5 (19.0; 49.9)	33.8 (22.5; 51.5)	0.28
RANTES [ng/ml]	23.9 (16.4; 33.0)	24.2 (16.8; 35.8)	0.58	25.2 (17.0; 36.8)	27.1 (14.9; 42.6)	0.73
adiponectin [ng/ml]	5191 (4042; 8662)	5387 (4414; 7830)	0.93	4954 (4042; 6236)	4991 (4414; 6095)	0.68
acute-phase-proteins/leukocyte count						
hsCRP [mg/l]	2.7 (0.8; 4.4)	2.1 (0.9; 5.5)	0.85	3.2 (0.8; 5.3)	2.0 (0.8; 3.6)	0.15
SAA [mg/l]	4.2 (3.0; 7.2)	5.4 (2.2; 9.0)	0.45	4.5 (3.5; 7.8)	4.9 (2.1; 7.6)	0.58
WBC count [per μl]	6250 (5750; 7250)	6400 (5400; 6600)	0.79	6250 (5950; 7350)	6400 (5400; 6450)	0.79

the analysis instead to those who lost at least 1 kg of body weight had no impact on the observed increase of IL-18, but attenuated the patient number further (n=9 out of 16 patients; data not shown).

Discussion

This prospective clinical study showed that a treatment with high-frequency EMS for 6 weeks reduced body weight and improved metabolic control in patients with type 2 diabetes. Moreover, the results remained almost stable in the subsequent weeks indicating that the treatment may also have longer-lasting effects.

hfEMS results in effective muscle contraction and produces less discomfort for the patient than other muscle stimulation methods like transcutaneous electrical nerve stimulation (TENS) using lower stimulation frequencies, which produce only superficial muscle contractions. EMS leads to muscle contraction of large motor units first with synchronous depolarisation and higher firing rates of their motoneurons, resulting in stronger muscle contraction than a voluntary contraction, but also in faster exhaustion and anaerobic metabolism of muscle (Requena et al., 2005). This "reverse-size" recruitment of motor units results in reduced shear forces between muscle fibres preventing discomfort or pain during treatment. Thus, the effect of voluntary isometric contraction can be mimicked by hfEMS treatment, and it is reasonable to hypothesise that comparable mechanisms, i.e. the reduction of insulin resistance, might be involved in mediating the effects of hfEMS on glucose metabolism. The effect on insulin sensitivity by voluntary physical activity is mediated via the increased expression of GLUT-4 on myocytes resulting in elevated glucose uptake and generation of glycogen (Hardin et al., 1995; Kennedy et al., 1999; Christ-Roberts et al., 2004). These effects are observed after acute as well as after chronic exercise, but it is not quite clear whether these effects are independent of an accompanying weight loss (Ross, 2003). An association between change in HbA1c and change in body weight is conceivable. However, we observed no correlation between these two variables after the treatment and therefore did not adjust for change in body weight in our analysis. In line with this, a reduction of insulin resistance measured by HOMA-IR after 6 weeks of treatment with hfEMS was observed, indicating that hfEMS increases insulin sensitivity of peripheral tissues, probably predominantly muscle tissue. This insulin sensitizing effect seems to be more pronounced with EMS treatment than after voluntary exercise (Hamada et al., 2004). Additionally, physical activity can overcome the impaired mitochondrial function observed in patients with type 2 diabetes and increases the amount of mitochondria in muscle of patients with diabetes (Toledo et al., 2007). These improvements are again closely associated with better metabolic control. Thus, hfEMS treatment mimics the beneficial effects of voluntary physical activity.

Despite the reduction of HbA1c fasting glucose levels remained virtually unchanged, suggesting a predominant effect of postprandial levels. Sufficient information from blood glucose diaries were not available and no standardised mixed meal test or oGTT was performed to address this potential effect but should be included in future studies.

Cytokines that are released by muscle tissue directly after exercise (Helge et al., 2003) seem to be related to carbohydrate metabolism of muscle and may induce an anti-inflammatory reaction. It has been shown that regular physical activity reduces the systemic levels of proinflammatory mediators like MCP-1 and IL-8 (Troseid et al., 2004; Kriketos et al., 2004). Although a slight increase of IL-18 levels was observed in our study, while IL-6 levels were reduced after hfEMS treatment at least in HbA1c responders, none of the changes reached statistical significance due to the relatively low sample size. However, it is conceivable that a longer duration of hfEMS treatment resulting in more pronounced weight loss and improvement of metabolic control might also be associated with an attenuation of subclinical inflammation.

To the best of our knowledge, only one study evaluated the effects of external muscle stimulation on metabolic control in patients with type 2 diabetes so far (Poole et al., 2005). They did not found

any improvement in body composition or glucose control. Possible reasons might be the low patient number (four diabetic patients) and the treatment method differing from ours as low-frequency external muscle stimulation was used, which results in only mild muscle contractions. Moreover, no control group was included in this study either, because the selection of an appropriate control group for EMS treatment is challenging and strongly depends on the research question, i.e. placebo treatment to analyse the efficiency of EMS treatment at all or a control treatment (voluntary exercise, isometric training, etc.) to address the equivalency of both treatments. Confounding by effects on the cardiorespiratory system by the control treatment need to be considered further. Thus, we refrained from designing a control group for this first larger proof-of-principle study as Poole et al. also did. Certainly, we cannot exclude a certain effect on glucose control and body weight by participation in the study per se ("study effect"). However, the effect of hfEMS treatment on HbA1c exceeds the effects observed in patients with type 2 diabetes after more than 28 days of participation in pharmacological intervention studies (Gale et al., 2007) indicating that the observed improvement of metabolic control is related to a true treatment effect. Nevertheless the observed improvements need to be evaluated using a larger sample with a carefully designed appropriate control treatment to overcome the limitations of our study.

In conclusion, this study has shown as a proof of principle study that high-frequency EMS has positive effects on body weight and metabolic control and appears to be a promising non-pharmacological additional treatment option for patients with type 2 diabetes.

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Conflict of interest: None.

References

1 Standards of medical care in diabetes. Diabetes Care 2007; 30 (Suppl 1): S4–S41
2 *Christ-Roberts CY*, *Pratipanawatr T*, *Pratipanawatr W*, *Berria R*, *Belfort R*, *Kashyap S*, *Mandarino LJ*. Exercise training increases glycogen synthase activity and GLUT4 expression but not insulin signaling in overweight nondiabetic and type 2 diabetic subjects. Metabolism 2004; 53: 1233–1242
3 *Gale EA*, *Beattie SD*, *Hu J*, *Koivisto V*, *Tan MH*. Recruitment to a clinical trial improves glycemic control in patients with diabetes. Diabetes Care 2007; 30: 2989–2992
4 *Hainaut K*, *Duchateau J*. Neuromuscular electrical stimulation and voluntary exercise. Sports Med 1992; 14: 100–113
5 *Hamada T*, *Hayashi T*, *Kimura T*, *Nakao K*, *Moritani T*. Electrical stimulation of human lower extremities enhances energy consumption, carbohydrate oxidation, and whole body glucose uptake. J Appl Physiol 2004; 96: 911–916
6 *Hardin DS*, *Azzarelli B*, *Edwards J*, *Wigglesworth J*, *Maianu L*, *Brechtel G*, *Johnson A*, *Baron A*, *Garvey WT*. Mechanisms of enhanced insulin sensitivity in endurance-trained athletes: effects on blood flow and differential expression of GLUT 4 in skeletal muscles. J Clin Endocrinol Metab 1995; 80: 2437–2446
7 *Helge JW*, *Stallknecht B*, *Pedersen BK*, *Galbo H*, *Kiens B*, *Richter EA*. The effect of graded exercise on IL-6 release and glucose uptake in human skeletal muscle. J Physiol 2003; 546: 299–305
8 *Herder C*, *Haastert B*, *Muller-Scholze S*, *Koenig W*, *Thorand B*, *Holle R*, *Wichmann HE*, *Scherbaum WA*, *Martin S*, *Kolb H*. Association of systemic chemokine concentrations with impaired glucose tolerance and type 2 diabetes: results from the Cooperative Health Research in the Region of Augsburg Survey S4 (KORA S4). Diabetes 2005; 54 (Suppl 2): S11–S17
9 *Herder C*, *Schneitler S*, *Rathmann W*, *Haastert B*, *Schneitler H*, *Winkler H*, *Bredahl R*, *Hahnloser E*, *Martin S*. Low-grade inflammation, obesity, and insulin resistance in adolescents. J Clin Endocrinol Metab 2007; 92: 4569–4574
10 *Houmard JA*, *Tanner CJ*, *Slentz CA*, *Duscha BD*, *MacCartney JS*, *Kraus WE*. Effect of the volume and intensity of exercise training on insulin sensitivity. J Appl Physiol 2004; 96: 101–106
11 *Huang H*, *Iida KT*, *Sone H*, *Ajisaka R*. The regulation of adiponectin receptors expression by acute exercise in mice. Exp Clin Endocrinol Diabetes 2007; 115: 417–422
12 *Karavidas AI*, *Raisakis KG*, *Parissis JT*, *Tsekoura DK*, *Adamopoulos S*, *Korres DA*, *Farmakis D*, *Zacharoulis A*, *Fotiadis I*, *Matsakas E*, *Zacharoulis A*. Functional electrical stimulation improves endothelial function and reduces peripheral immune responses in patients with chronic heart failure. Eur J Cardiovasc Prev Rehabil 2006; 13: 592–597
13 *Kennedy JW*, *Hirshman MF*, *Gervino EV*, *Ocel JV*, *Forse RA*, *Hoenig SJ*, *Aronson D*, *Goodyear LJ*, *Horton ES*. Acute exercise induces GLUT4 translocation in skeletal muscle of normal human subjects and subjects with type 2 diabetes. Diabetes 1999; 48: 1192–1197
14 *Kirk A*, *Mutrie N*, *MacIntyre P*, *Fisher M*. Increasing physical activity in people with type 2 diabetes. Diabetes Care 2003; 26: 1186–1192
15 *Kolb H*, *Mandrup-Poulsen T*. An immune origin of type 2 diabetes? Diabetologia 2005; 48: 1038–1050
16 *Kriketos AD*, *Gan SK*, *Poynten AM*, *Furler SM*, *Chisholm DJ*, *Campbell LV*. Exercise increases adiponectin levels and insulin sensitivity in humans. Diabetes Care 2004; 27: 629–630
17 *Krug LM*, *Haire-Joshu D*, *Heady SA*. Exercise habits and exercise relapse in persons with non-insulin-dependent diabetes mellitus. Diabetes Educ 1991; 17: 185–188
18 *Levy JC*, *Matthews DR*, *Hermans MP*. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. Diabetes Care 1998; 21: 2191–2192
19 *Lindstrom J*, *Louheranta A*, *Mannelin M*, *Rastas M*, *Salminen V*, *Eriksson J*, *Uusitupa M*, *Tuomilehto J*. The Finnish Diabetes Prevention Study (DPS): Lifestyle intervention and 3-year results on diet and physical activity. Diabetes Care 2003; 26: 3230–3236
20 *Muller S*, *Martin S*, *Koenig W*, *Hanifi-Moghaddam P*, *Rathmann W*, *Haastert B*, *Giani G*, *Illig T*, *Thorand B*, *Kolb H*. Impaired glucose tolerance is associated with increased serum concentrations of interleukin 6 and co-regulated acute-phase proteins but not TNF-alpha or its receptors. Diabetologia 2002; 45: 805–812
21 *Petersen AM*, *Pedersen BK*. The anti-inflammatory effect of exercise. J Appl Physiol 2005; 98: 1154–1162
22 *Poole RB*, *Harrold CP*, *Burridge JH*, *Byrne CD*, *Holt RI*. Electrical muscle stimulation acutely mimics exercise in neurologically intact individuals but has limited clinical benefits in patients with type 2 diabetes. Diabetes Obes Metab 2005; 7: 344–351
23 *Reichstein L*, *Labrenz S*, *Ziegler D*, *Martin S*. Effective treatment of symptomatic diabetic polyneuropathy by high-frequency external muscle stimulation. Diabetologia 2005; 48: 824–828
24 *Requena SB*, *Padial PP*, *Gonzalez-Badillo JJ*. Percutaneous electrical stimulation in strength training: an update. J Strength Cond Res 2005; 19: 438–448
25 *Ross R*. Does exercise without weight loss improve insulin sensitivity? Diabetes Care 2003; 26: 944–945
26 *Ross R*, *Dagnone D*, *Jones PJ*, *Smith H*, *Paddags A*, *Hudson R*, *Janssen I*. Reduction in obesity and related comorbid conditions after diet-induced weight loss or exercise-induced weight loss in men. A randomized, controlled trial. Ann Intern Med 2000; 133: 92–103
27 *Smith JK*, *Dykes R*, *Douglas JE*, *Krishnaswamy G*, *Berk S*. Long-term exercise and atherogenic activity of blood mononuclear cells in persons at risk of developing ischemic heart disease. JAMA 1999; 281: 1722–1727
28 *Toledo FG*, *Menshikova EV*, *Ritov VB*, *Azuma K*, *Radikova Z*, *DeLany J*, *Kelley DE*. Effects of physical activity and weight loss on skeletal muscle mitochondria and relationship with glucose control in type 2 diabetes. Diabetes 2007; 56: 2142–2147
29 *Troseid M*, *Lappegard KT*, *Claudi T*, *Damas JK*, *Morkrid L*, *Brendberg R*, *Mollnes TE*. Exercise reduces plasma levels of the chemokines MCP-1 and IL-8 in subjects with the metabolic syndrome. Eur Heart J 2004; 25: 349–355
30 *Walker KZ*, *Piers LS*, *Putt RS*, *Jones JA*, *O'Dea K*. Effects of regular walking on cardiovascular risk factors and body composition in normoglycemic women and women with type 2 diabetes. Diabetes Care 1999; 22: 555–561

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Muskelstimulation mit hoch-frequenter externer Muskelstimulation mindert signifikant die Symptome der diabetischen Polyneuropathie

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Fragestellung: Diabetische Neuropathie ist eine der häufigsten chronischen Komplikationen bei Diabetes mellitus (DM). Typische Symptome sind Kribbeln, Brennen, Taubheitsgefühl oder Schmerzen in Füßen und Unterschenkeln, die die Lebensqualität stark beeinträchtigen. Medikamentöse Therapien können Schmerzen reduzieren, haben jedoch häufig Nebenwirkungen. In einer Pilotstudie konnten wir zeigen, dass hoch-frequente externe Muskelstimulation (hfEMS) neuropatische Symptome effektiver reduziert als transkutane elektrische Nervenstimulation. Es war daher das Ziel den Einfluss von hfEMS auf neuropatische Symptome in einer größeren Kohorte unter realen Alltagsbedingungen zu untersuchen.

Methodik: Durch Pressemitteilungen wurden auf die Studie aufmerksam gemacht. Patienten, die sich im Studienzentrum meldeten und die Einschluss- (DM und symptomatische diabetische Polyneuropathie) und keines der Ausschlusskriterien (Herzschrittmacher, Chemotherapie) erfüllten, wurde ein Gerät zur hfEMS (HiToP®191, gbo Medizintechnik AG) zugeschickt mit der Anleitung, es mindestens 4 mal pro Woche für 30min über einen Zeitraum von 4 Wochen zu verwenden. Die Intensität und Häufigkeit der vorhandenen Symptome wurden in standardisierten Telefongesprächen auf einer Skala von 0–10 (inkl. Neuropathie Symptom Score (NSS)) vor Beginn der Therapie (t0), nach 8 Tagen (t1) und 4 Wochen (t2), sowie 3 Wochen nach Ende der Therapie (t3) erfasst. Patienten, deren neuropatische Symptome sich um mindestens 3 Skalenpunkte verbessert hatten (t2 vs. t0), wurden als Therapieresponder gewertet.

Ergebnisse: In der Kohorte (n=167; 132Männer; Alter 67,3±9,2 Jahre; DM-Dauer 12,7±9,0 Jahre; HbA1c 7,0±1,2%; NSS 6,8±2,0) kam es durch Anwendung von

hfEMS zu einer Verringerung der Intensität (und Häufigkeit) des Kribbelns (3,4±2,4 (t2) vs. 5,6±2,7 (t0); p<0,0001), Brennens (2,8±2,5 vs. 4,4±3,2; p<0,0001), der Schmerzen (3,0±2,6 vs. 5,1±3,2; p<0,0001) und des Taubheitsgefühls (4,1±2,8 vs. 6,5±3,0; p<0,0001) in Füßen (und Unterschenkeln) wie auch bei der Schlafbeeinträchtigung (2,6±2,4 vs. 4,8±3,3; p<0,0001). Interessanterweise traten auch 3 Wochen nach Beendigung der Therapie die Symptome schwächer und seltener (p<0,0001) im Vergleich zu vorher auf (t3 vs. t0), wobei allerdings bereits eine Verschlechterung im Vergleich zum Zeitpunkt t2 zu beobachten war (p<0,0001). Darüber hinaus sprachen 82% der Patienten als Therapieresponder auf hfEMS an. Dies war assoziiert mit einer längeren DM-Dauer (r=0,18, p=0,02), höherem NSS (r=0,16, p=0,04) und HbA1c (r=0,20, p=0,02) zu Therapiebeginn.

Schlussfolgerungen: hfEMS verbessert signifikant die Symptome der diabetischen Polyneuropathie und hat möglicherweise auch längerfristige Effekte, da Symptome auch nach Beendigung der Therapie weniger intensiv und weniger häufig auftreten. Da hfEMS keine Nebenwirkungen hat und vorrangig Patienten mit weiter fortgeschrittenem DM darauf ansprechen, sollte hfEMS als Therapie Patienten mit diabetischer Neuropathie angeboten werden.

High-frequency External Muscle Stimulation significantly improves Symptomatic Diabetic Polyneuropathy

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Background

Diabetic neuropathy is one of the most common chronic complications of diabetes mellitus (DM). In the majority of cases it is associated with prickle, burning, numbness or pain in feet or lower legs resulting in extensive impairments in daily life. Therapies mainly focus on the reduction of pain by drugs which often have severe side-effects. In a pilot trial we demonstrated that high-frequency external muscle stimulation (hfEMS) is able to reduce neuropathic symptoms more effectively than transcutaneous electrical nerve stimulation. Thus, we aimed to assess the impact of hfEMS on neuropathic symptoms in a larger cohort in a real world study design.

Subjects and Methods

Patients with symptomatic diabetic neuropathy, who called the study centre and who fulfilled all inclusion (DM and symptomatic diabetic neuropathy) and no exclusion criteria (heart pacemaker, history of chemotherapy) were sent an hfEMS device (HiToP®191 gbo Medizintechnik AG) with instructions to use it at least 4 times/week for 30 min over a period of 4 weeks (**Fig.1**).

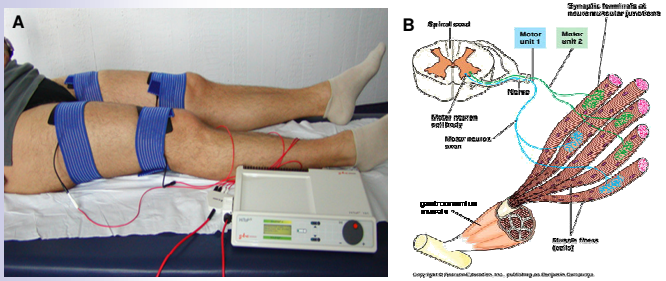

Fig.1 High-frequency external muscle stimulation. (**A**) Electrodes are placed at the upper leg. (**B**) Frequency modulation between 4 and 32 kHz stimulates large efferent axons, which are localized under the surface of the quadriceps femoris. These efferent axons innervate large motor units, which mainly consist of fast-twitch, glycolytic active, more fatigable muscle fibers.

Symptoms were assessed on a scale from 1-10 at standardized telephone calls before hfEMS therapy (including 5-item neuropathy symptom (NS) score) , after the 1st week and 4th week of treatment as well as 3 weeks after the end of treatment. Patients, whose neuropathic symptoms improved in sum (4th week vs. before) were classified as hfEMS responders.

Literature

- **Reichstein et al.** (2005): Effective treatment of symptomatic diabetic polyneuropathy by high-frequency external muscle stimulation. *Diabetologia* 48: 824-8
- **Rose et al.** (2008): Beneficial effects of external muscle stimulation on glycaemic control in patients with type 2 diabetes. *Exp Clin Endocrinol Diabetes* (in press)

Results

Table 1 Clinical characteristics

	Study population (n=414)
Sex [n] *men / women*	307 (74,2%) / 107 (25,8%)
Age [years]	67 ± 9
Duration of DM [years]	14 ± 10
DM treatment [n] *none / OAD / insulin*	16 (4%) / 120 (29%) / 274 (66%)
HbA1c [%]	7,1 ± 1,1
DM type [n] *T1DM / T2DM*	17 (4%) / 393 (95%)
NS score [points]	6,9 ± 1,9

DM, diabetes mellitus; OAD, oral antidiabetic drugs; HbA1c, glycosylated hemoglobin A1c; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; NS score, neuropathy symptom score.

hfEMS significantly improved the intensity (and frequency) of pain, burning, prickle and numbness in feet (and lower legs) as well as impairments during sleep. Interestingly, 3 weeks after therapy symptoms remained less intense and less frequent compared to baseline but were significantly higher than after the 4th week of treatment (**Fig.2**).

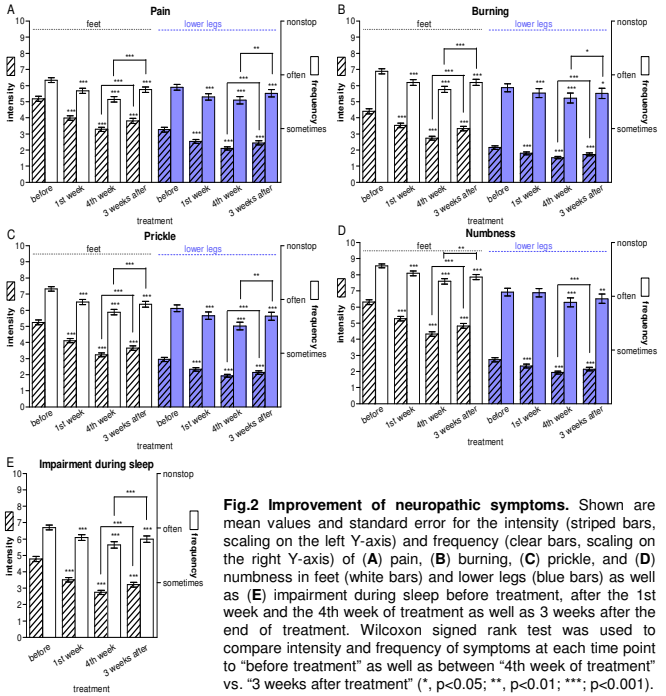

Fig.2 Improvement of neuropathic symptoms. Shown are mean values and standard error for the intensity (striped bars, scaling on the left Y-axis) and frequency (clear bars, scaling on the right Y-axis) of (**A**) pain, (**B**) burning, (**C**) prickle, and (**D**) numbness in feet (white bars) and lower legs (blue bars) as well as (**E**) impairment during sleep before treatment, after the 1st week and the 4th week of treatment as well as 3 weeks after the end of treatment. Wilcoxon signed rank test was used to compare intensity and frequency of symptoms at each time point to "before treatment" as well as between "4th week of treatment" vs. "3 weeks after treatment" (*, p<0.05; **, p<0.01; ***; p<0.001).

88.4% of patients were identified as hfEMS responders. Positive response to hfEMS was associated with female sex, higher HbA1c and higher NS score at baseline (**Fig.3**). After adjustment only the NS score remained predictive for improvement of symptoms (**Table 2**).

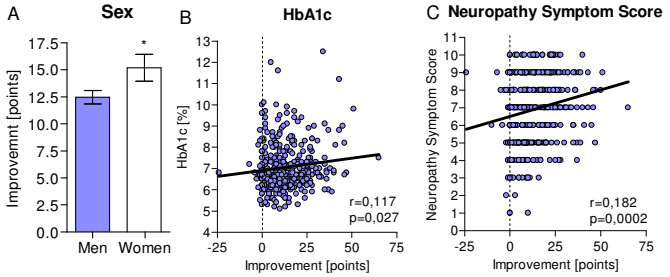

Fig.3 Association between improvement of neuropathic symptoms and baseline clinical parameters. Shown are (**A**) the summed up mean improvement and standard error of the intensity of neuropathic symptoms. Mann Whitney test was used to compare differences between men and women (*, p<0.05). Improvement of symptoms was assessed by summing up the difference for the nine measured symptoms, i.e. intensity of pain, burning, prickle, numbness in feet and lower legs as well as impairment during sleep "at the 4th week of treatment" vs. "before treatment". Associations between the improvements of symptoms and clinical parameters as (**B**) HbA1c and (**C**) neuropathy symptom score at baseline were analyzed by Pearson regression.

Table 2 Association between improvement of symptoms and clinical data.

	Linear regression: improvement (points)		Logistic regression: improvement (yes / no)			
	p	p*	OR	CI	OR*	CI*
Sex	**0.036**	0.077	1.37	0.66-2.86	1.03	0.56-3.00
Age	0.960	0.612	0.98	0.95-1.02	0.98	0.94-1.02
DM duration	0.247	0.288	0.98	0.95-1.01	1.00	0.97-1.04
DM treatment	0.551	0.451	**0.50**	**0.25-0.98**	0.65	0.31-1.38
HbA1c	**0.027**	0.060	1.03	0.76-1.39	1.12	0.79-1.59
DM type	0.432	0.326	1.66	0.46-6.00	2.12	0.52-8.67
NS Score	**0.0002**	**0.0001**	**1.20**	**1.03-1.40**	**1.23**	**1.04-1.46**

Odds ratios (OR) and confidence intervals (CI) were determined by logistic regression without or with adjustment to sex, age, duration of diabetes mellitus (DM), DM medication, hemoglobin A1c (HbA1c), diabetes type and neuropathy symptom (NS) score.

Conclusions

hfEMS significantly improves discomfort and pain associated with diabetic neuropathy within 4 weeks of treatment. As symptoms remain less intense and less frequent 3 weeks after the end of treatment, although they increase again, a continuous treatment is suggested. As hfEMS treatment has no side-effects and is most effective in patients with more severe symptoms we suggest to offer hfEMS therapy to all patients with diabetic neuropathy.

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External Electric Muscle Stimulation Improves Burning Sensations and Sleeping Disturbances in Patients with Type 2 Diabetes and Symptomatic Neuropathy

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ABSTRACT

Objective. External muscle stimulation (EMS) of the thighs was previously shown to have beneficial effects in a pilot study on painful diabetic neuropathy. However, differential effects on specific symptoms of neuropathy as well as determinants of treatment response have not been described.

Design. Ninety-two type 2 diabetes patients with different neuropathic symptoms were included in a prospective uncontrolled trial. Patients were treated twice a week for 4 weeks. Symptoms were graded on numeric scales at baseline, before the second and the eighth visit.

Results. Seventy-three percent of the participants reported marked improvement of symptoms. Subjective treatment response was positively and independently associated with symptom intensity but independent of disease extent, metabolic factors, age, or gender. Total symptoms graded by patients on numerical scales decreased significantly after 4 weeks of treatment. Patients in the upper tertile of symptom intensity showed significant improvement of paresthesia, pain, numbness and most pronounced for burning sensations and sleeping disturbances.

Conclusions. In an uncontrolled setting, EMS seems to be an effective treatment for symptomatic neuropathy in patients with type 2 diabetes, especially in patients with strong symptoms.

Key Words. Type 2 Diabetes; Diabetic Neuropathy; Electric Muscle Stimulation; Treatment

Introduction

It was recently shown in a pilot study that external electric muscle stimulation (EMS) of the thigh causing isometric muscle contraction might be an effective treatment for painful diabetic neuropathy [1]. Type 1 and 2 diabetes patients were treated with EMS and compared with patients treated with transcutaneous electric nerve stimulation (TENS) [1,2]. Both EMS and TENS reduced the total symptom score significantly, while EMS resulted in a significantly higher response rate [1]. So far, there is no information regarding the influences of EMS on neuronal function and mechanisms underlying the observed treatment effects. Previous studies suggested that electrical stimulation activates the dorsal columns, inhibits C-fibers, and consequently leads to a decrease in pain perception [3]. EMS, which can stimulate a large number of nerves in the thigh, might therefore lead to a spinal stimulation that in turn decreases excitability of small nerve fibers.

It is not known which specific symptoms of diabetic neuropathy can be treated using EMS and which factors determine the treatment responses. Hence, we conducted this study in a large group of type 2 diabetes patients with neuropathic symptoms to identify subjects benefiting from this intervention and studied effects on different aspects of symptomatic neuropathy.

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Table 1 Baseline characteristics of the treated patients (N = 92)

Age (years)	66 ± 8
Gender (m/f)	64/28
Diabetes duration (years)	13 ± 9
BMI (kg/m^2)	31 ± 5
HbA1c (%)	6.8 ± 8
Total cholesterol (mg/dL)	190 ± 37
Creatinine (mg/dL)	1.0 ± 0.3
Urea (mg/dL)	42 ± 15
GPT (U/l)	26 ± 14
GOT (U/l)	34 ± 21
Neuropathy symptom scores	7.6 ± 1.3
Neuropathy disability scores	6.1 ± 2.3
Insulin therapy (%)	49
Oral medication (%)	67
Previous or current pain medication (%)	59
Anticonvulsants	34
Antidepressants	4
Opioids	7
Antioxidants	17
Others	9
Treatment response individual judgment (%)	
≥30% reduction in mean	73
Symptom score (N = 81)	47

GPT = glutamat-pyruvat-transaminase; GOT = glutamat-oxalazetat-transaminase.

Methods

Participants

One hundred type 2 diabetes patients with symptomatic diabetic neuropathy were included in this study after giving written informed consent. As a consequence of a lack in suitable placebo treatments, the study was performed in a prospective uncontrolled design. The study protocol was approved by the University of Heidelberg Ethics Committee. Patients were eligible if they reported any symptoms of diabetic neuropathy (i.e., pain, paresthesia, burning sensations, numbness, sleeping disturbance). Patients with implanted pacemakers or defibrillators were excluded from the study. Eight patients were excluded from the analyses as other causes of neuropathy or symptoms could not be excluded (i.e., excessive alcohol consumption, peripheral artery disease and ischemia, chemotherapy, amyloidosis). The detailed characteristics of the patients included are shown in Table 1.

Grading of Symptoms

At baseline, diabetic neuropathy was graded using the established neuropathy disability scores (NDS) and neuropathy symptom scores (NSS) [4]. In addition, patients had to grade their symptoms on 10-point numeric scales (1 = no symptoms, 10 = worst possible) as previously reported [1] at baseline, before the second and eighth treatment session. The duration of treatment effects was estimated by the participants before the eighth session. Eighty-one participants completed the 4-week protocol while 11 patients were lost to follow-up after the fourth session.

External Electric Muscle Stimulation

Patients were treated with EMS for 60 minutes, twice a week, and for 4 weeks (eight treatment sessions). EMS was performed using the HiToP® 184 device (gbo Medizintechnik, Rimbach, Germany). As previously described [1], a 20-Hz frequency scan of carrier frequencies between 4,096 Hz and 32,768 Hz was used to generate a deep and comfortable muscle contraction. This application was repetitively modulated with 3 seconds rest time, 3 seconds rise time, and 3 seconds contraction time. The intensity of the electrical stimulation was adjusted to a tolerable level causing muscle contraction and avoiding pain or discomfort.

Statistical Procedures

Logistic regression models were calculated to detect possible influence factors on treatment response. In these models, patients were divided into responders and non-responders according to self-report of improvement of symptoms and in patients completing the 8-week protocol according to a minimum of 30% mean decrease in symptoms according to the graded symptom scores before the eighth session.

A total symptom score (TSS) was calculated by addition of the individual scores on the numeric scales, leading to a maximum score of 50. To study influences of EMS on the specific symptoms, patients were divided into tertiles of the TSS and the respective symptoms (i.e., paresthesia, pain, burning sensation, numbness, sleeping disturbance). Tertiles of patients and the respective symptom scores for equally powered groups were as follows: paresthesia (1–4/5/6–10), pain (1–3/4–6/7–10), burning sensation (1–3/4–6/7–10), sleeping disturbance (1–3/4–5/6–10), and numbness (1–3/4–6/7–10). Treatment effects were studied in the entire group and in patients in the highest tertile of the respective score. Significant differences in symptom scores between the follow-up measurements were determined using two-tailed paired *t*-tests compared with the baseline. All statistical analyses were performed using SPSS (Version 15.0).

Results

Sixty-seven (~73%) of the 92 participants reported subjective improvement of neuropathic symptoms. Eighty-one patients completed the protocol, while 11 patients did not continue after the fourth treatment session; all of these patients reported no treatment response. Forty-seven percent of the participants completing the protocol had an improvement of the mean symptom score by ≥30% on the graded symptom scales. Although patients were not remunerated for participation or travel expenses, adherence to the protocol was 100% in the patients reporting improvement of symptoms. The mean duration of symptomatic relief was 31 ± 21 hours; the maximum duration reported was 80 hours. Fifty-four patients (~59%) were previously or currently treated with medication for neuropathic symptoms and participants currently treated continued medication; the number of patients with an improvement of the mean symptom score by ≥30% was similar in these patients compared with previously untreated participants (41% vs 51%, P = ns). The only side effect of EMS reported was mild muscle soreness in the thighs on the day after treatment.

Differences between responders and non-responders were studied at baseline to identify possible influence factors on treatment responses. Responders according to self-judgment had more intense symptoms as given by the NSS scores (7.8 ± 1.2 vs 7.2 ± 1.5, $P = 0.04$), and there was a trend toward older age in non-responders (65 ± 8 vs 69 ± 8, $P = 0.07$). When patients were divided into responders and non-responders by a minimum of 30% decrease in mean symptom score as was previously suggested [5], there were no significant differences in the baseline characteristics (not shown). Logistic multivariate models including biometrical data and the classical risk factors revealed the NSS to be the only variable independently associated with subjective treatment response ($\beta = -0.47$, $P = 0.02$, Table 2). In participants with a minimum decrease of 30% in mean symptom score after 4 weeks of treatment, there was only a trend for an association with the NSS (Table 2).

When all participants in this study were analyzed, the TSS significantly improved on visit 8 (25.7 ± 10.5 vs 19.2 ± 10.4, $P < 0.001$), while the change in TSS on visit 2 was not significant (26.0 ± 10.3 vs 24.8 ± 10.6, P = ns). In the self-reported responders, TSS improved marginally significant on visit 2 (25.9 ± 10.4 vs 24.4 ± 10.7, $P = 0.05$) and highly significant on visit 8 (26.0 ± 10.4 vs 18.2 ± 10.4, $P < 0.001$). In self-reported non-responders, TSS did not change significantly on visit 2 (26.3 ± 10.3 vs 26.0 ± 10, P = ns) and visit 8 (24.2 ± 11.2 vs 23.5 ± 9.7, P = ns). When all patients were divided into tertiles of TSS, the second and third tertiles showed improvements of TSS on visits 2 and 8, while the lower tertile showed significant improvement of TSS on visit 8 only (Figure 1a). In self-reported responders, all three tertiles of TSS showed significant improvements of TSS on visit 8 only (Figure 2b).

Table 2 Logistic multivariate analyses of variables influencing response to treatment

	Regression Coefficient β (SE)	Regression Coefficient β (SE)
Age	–0.05 (0.04)	–0.02 (0.03)
Gender	–0.52 (0.59)	–0.12 (0.63)
Diabetes duration	–0.03 (0.03)	0.04 (0.03)
BMI	–0.05 (0.06)	–0.01 (0.05)
HbA1c	0.44 (0.20)	–0.45 (0.32)
Neuropathy symptom scores	0.47 (0.20)*	0.37 (0.21)**
Neuropathy disability scores	–0.18 (0.12)	0.69 (1.46)
Creatinine	0.33 (1.02)	–0.25 (0.91)

* $P = 0.02$; ** $P = 0.08$.
Response to treatment is judged as reported by the individual patient (left column) and in patients with a ≥30% reduction of mean symptoms on the feet after 4 weeks of treatment (right column, N = 81).
Treatment response is the dependent variable and coded numerically as 1 = non-responder and 2 = responder.

Changes in specific symptoms were studied in all participants followed up to the eighth visit and patients in the upper tertile of the respective symptom score on visits 2 and 8 (Figure 2, Table 3). The mean score of all symptoms (Figure 2a), paresthesia (Figure 2b), pain (Figure 2c), burning sensations (Figure 2d), sleeping disturbances (Figure 2e), and even numbness (Figure 2f) improved significantly on visit 2 and visit 8. The treatment effects were strongest on visit 8 for burning sensations (8.5 ± 1.2 vs 4.9 ± 2.5, $P < 0.001$, Table 3) and sleeping disturbances (7.9 ± 1.4 vs 4.6 ± 2.8, $P < 0.001$, Table 3) as documented on the 10-point scale. The relative decrease in severity of symptoms for these scores was approximately –42%.

Discussion

This is the first study on treatment effects of EMS in a large group of type 2 diabetes patients. The data show significant improvement of total symptoms and each specific quality of symptoms. Sig-

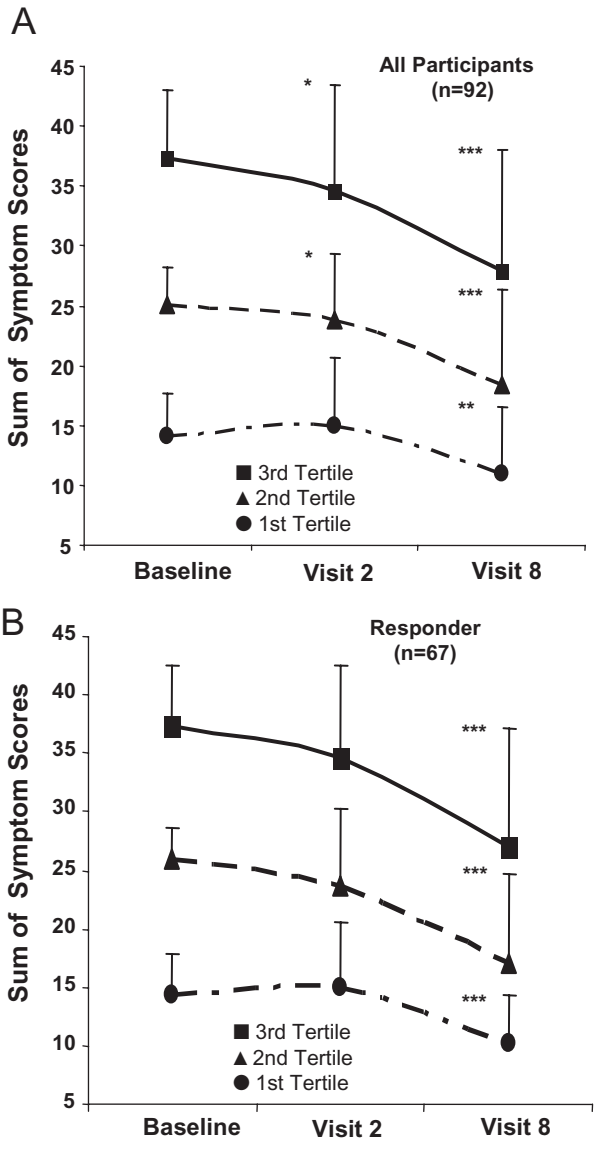

Figure 1 (A) Total symptom score (TSS) in all participants of the study by tertile of TSS. Patients in the upper and middle tertile of TSS showed significant reductions of symptoms on the second and the eighth visit. Patients in the lower tertile showed significant reductions on visit 8 only. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs TSS at baseline. (B) When responders were analyzed separately, highly significant reductions in total symptoms were observed on visit 8 only. This was true for type 2 diabetes patients in all three tertiles of the TSS.

nificant improvement was found in all tertiles of the TSS; however, the treatment effects were most pronounced in the upper tertile of patients affected by burning sensations and sleeping disturbances after 4 weeks of treatment. In these patients, symptoms were reduced by ~42%. This reduction in symptoms can be considered a strong and clinically relevant improvement, which was previously shown in a meta-analysis of studies comparing placebo-controlled data on pain reduction detected using numerical scales and in patients with different causes of neuropathic pain [6].

Multivariate analyses revealed that response to treatment was independently and positively associated with the NSS, but not with the NDS. This suggests, that type 2 diabetes patients at all ages and stages of disease might benefit from EMS. Additional metabolic factors such as BMI or even HbA1c, as a marker of long-term glucose control, did not influence the response rate significantly (Table 2). There were no significant differences in response rates between patients previously treated with pharmacological interventions and treatment-naive participants. This indicates that EMS might even be effective in patients that are not sufficiently treated using conventional medications such as anticonvulsants and antidepressants.

Pain and paresthesia in our study were reduced by ~31% and ~35%, respectively, effects that can also be considered clinically relevant [6]. It is likely that a significant part of the treatment effects can be attributed to placebo effects; the reduction of numbness was calculated at ~24% and is likely to represent the placebo effect in our study. Yet definite differentiation of placebo and treatment effects need to be clarified in future placebo-controlled trials.

Our data support the previously published high response rates of EMS in pilot studies and patients with symptomatic diabetic and uremic neuropathy [1,7] and further define the duration of treatment effects as well as specific symptoms that can be treated efficiently. The mean duration of the treatment effect was limited to 31 hours and varied strongly between participants. The underlying reasons for this large variation remain unclear, and it seems important to decipher the physiological changes induced by EMS to understand this finding. Although not an endpoint of this study, it seems likely that for example the significant reduction of sleeping disorders reported by the participants leads to a major improvement in quality of life. This improvement and the lack of severe adverse events seem to be reflected by the strong adherence to the study protocol (100% in self-reported responders) over a period of 4 weeks and eight treatment sessions. Future controlled studies will be needed to study cost-effectiveness and clinical efficacy of EMS in comparison to pharmaceutical interventions, especially in consideration of missing relevant side effects. In addition, study

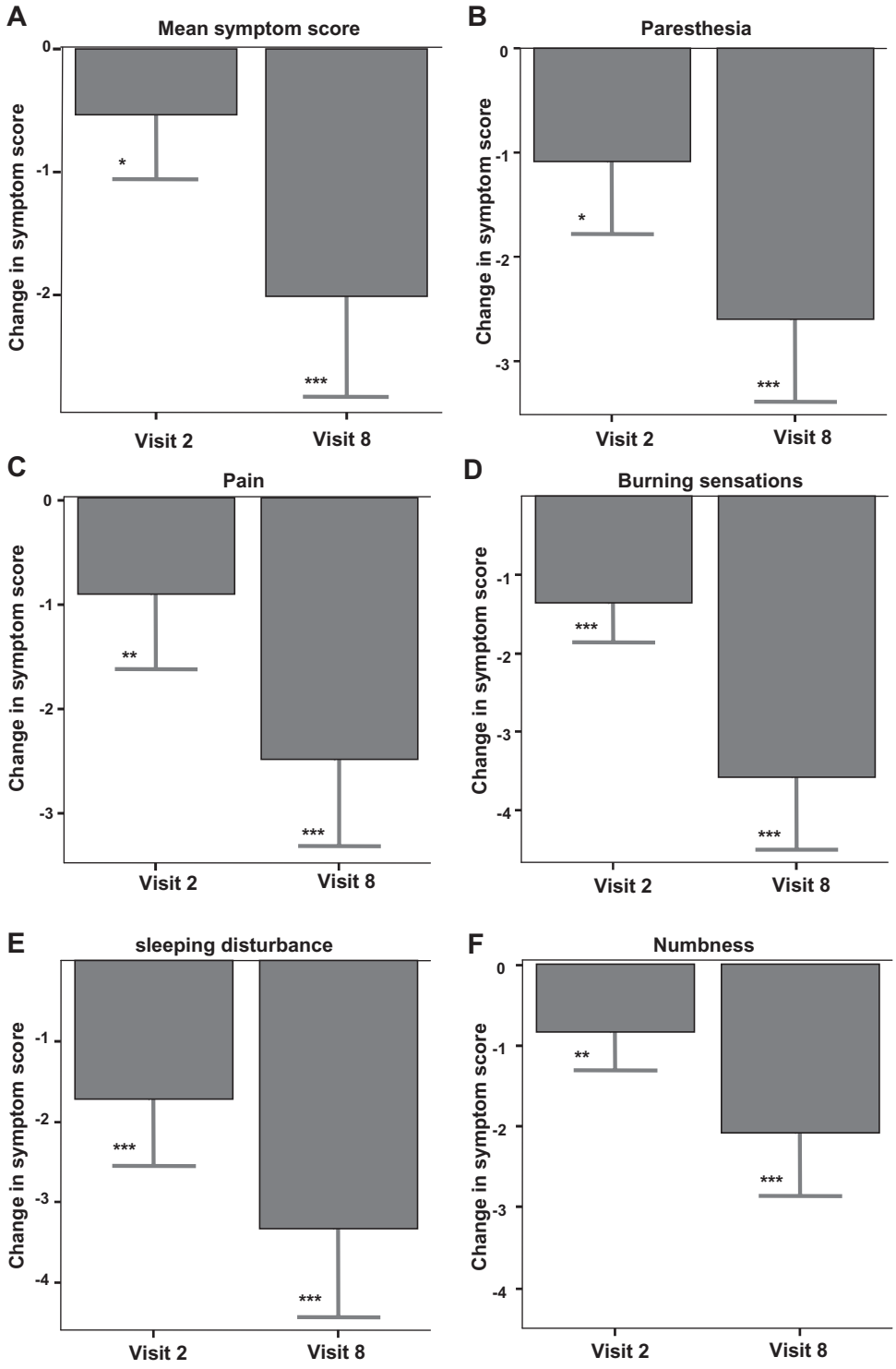

Figure 2 Treatment effects of external muscle stimulation (EMS) on symptoms of diabetic neuropathy were analyzed in participants in the upper tertile of the respective scores to study patients that are significantly affected by the specific symptom. (A) Mean symptom score, (B) paresthesia, (C) pain, (D) burning sensation, (E) sleeping disturbances, and (F) even numbness were significantly improved on visit 2 and visit 8. Treatment effects were strongest for the reduction of burning sensations and sleeping disturbances (D, E). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs the respective symptom score at baseline. Data are given as the mean change in symptom score ± SD.

Table 3 Symptom scores at baseline and on the eighth visit for all patients followed up (N = 81) and patients in the upper tertile of the respective baseline symptom score

	All Patients Followed Up (N = 81)		Patients in the Upper Tertile of Symptom Scores		
	Baseline	8th visit	Baseline	8th visit	(N)
Mean	5.1 ± 2.1	3.8 ± 2.1**	7.6 ± 1.1	5.6 ± 2.0**	(26)
Paresthesia	5.2 ± 2.4	3.8 ± 2.4**	7.5 ± 1.5	4.9 ± 2.2**	(30)
Pain	5.1 ± 3.0	3.7 ± 2.7**	8.3 ± 1.0	5.7 ± 2.4**	(31)
Burning sensation	5.2 ± 3.1	3.7 ± 2.4**	8.5 ± 1.2	4.9 ± 2.5**	(29)
Sleeping disturbances	4.8 ± 2.8	3.4 ± 2.5**	7.9 ± 1.4	4.6 ± 2.8**	(30)
Numbness	5.4 ± 3.1	4.6 ± 2.8*	8.6 ± 1.1	6.5 ± 2.4**	(33)

* $P < 0.01$; ** $P < 0.001$ as given by paired *t*-test.
(N) is the number of patients in the upper tertile of the respective baseline symptom score that were followed up to visit 8.
Data are given as mean ± standard deviation.

protocols will have to include measures of depression as additional endpoint which is known to have important associations with symptomatic neuropathy and treatment effects [8].

In the lack of a control group, numbers needed to treat can be estimated utilizing placebo data from previously published studies on tricyclic antidepressants and traditional anticonvulsants in which a moderate relief of pain by 30% was considered as the cut-off for treatment response [5]. In these published studies [9–12], 4 out of 81 patients on placebo treatment experienced a moderate ≥30% symptom relief compared with 38 out of the 81 patients treated with EMS in this study. This would correspond to a number needed to treat of 2.4 in our study and argue for EMS being a very effective treatment option. However, these considerations remain speculative and treatment effects need to be clarified in future placebo-controlled trials. A specific placebo device that causes superficial skin stimulation without influences on muscle and nerves was not available for this study and will have to be constructed for this purpose.

In conclusion, EMS was shown to be of some benefit in ~40–70% of the type 2 diabetes patients treated depending on the definition of treatment response (i.e., ≥30% reduction in mean symptom score or self-reported), is free of significant side effects, and led to an accentuated improvement in burning sensations and sleeping disorders. Forty-one percent of patients currently or previously treated with pain medication had an improvement of mean symptoms by ≥30%. This data have to be judged in view of the results obtained in clinical trials of pharmaceutical interventions that resulted in numbers needed to treat of ~2–4 and led to significant side effects [13–16]. This study was performed in an uncontrolled design. Future controlled trials including pharmaceutical interventions will be needed to clearly define the clinical and economic efficacy of this treatment option.

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References

1 Reichstein L, Labrenz S, Ziegler D, Martin S. Effective treatment of symptomatic diabetic polyneuropathy by high-frequency external muscle stimulation. Diabetologia 2005;48:824–8.
2 Kumar D, Marshall HJ. Diabetic peripheral neuropathy: Amelioration of pain with transcutaneous electrostimulation. Diabetes Care 1997;20:1702–5.
3 Watkins ES, Koeze TH. Spinal cord stimulation and pain relief. BMJ 1993;307:462.
4 Young MJ, Boulton AJ, MacLeod AF, Williams DR, Sonksen PH. A multicentre study of the prevalence of diabetic peripheral neuropathy in the United Kingdom hospital clinic population. Diabetologia 1993;36:150–4.
5 Wong MC, Chung JW, Wong TK. Effects of treatments for symptoms of painful diabetic neuropathy: Systematic review. BMJ 2007;335:87.
6 Farrar JT, Young JP Jr, LaMoreaux L, Werth JL, Poole RM. Clinical importance of changes in chronic pain intensity measured on an 11-point numerical pain rating scale. Pain 2001;94:149–58.
7 Klassen A, Di Iorio B, Guastaferro P, et al. High-tone external muscle stimulation in end-stage renal disease: Effects on symptomatic diabetic and uremic peripheral neuropathy. J Ren Nutr 2008;18:46–51.
8 Vileikyte L, Leventhal H, Gonzalez JS, et al. Diabetic peripheral neuropathy and depressive symptoms: The association revisited. Diabetes Care 2005;28:2378–83.
9 Kochar DK, Jain N, Agarwal RP, et al. Sodium valproate in the management of painful neuropathy in type 2 diabetes—A randomized placebo controlled study. Acta Neurol Scand 2002;106:248–52.

10 Max MB, Culnane M, Schafer SC, et al. Amitriptyline relieves diabetic neuropathy pain in patients with normal or depressed mood. Neurology 1987; 37:589–96.
11 Max MB, Kishore-Kumar R, Schafer SC, et al. Efficacy of desipramine in painful diabetic neuropathy: A placebo-controlled trial. Pain 1991;45:3–9, discussion 1–2.
12 Rull JA, Quibrera R, Gonzalez-Millan H, Lozano Castaneda O. Symptomatic treatment of peripheral diabetic neuropathy with carbamazepine (Tegretol): Double blind crossover trial. Diabetologia 1969;5: 215–8.
13 Saarto T, Wiffen PJ. Antidepressants for neuropathic pain. Cochrane Database Syst Rev 2005: CD005454.
14 Wiffen P, Collins S, McQuay H, et al. Anticonvulsant drugs for acute and chronic pain. Cochrane Database Syst Rev 2005:CD001133.
15 Wiffen PJ, McQuay HJ, Edwards JE, Moore RA. Gabapentin for acute and chronic pain. Cochrane Database Syst Rev 2005:CD005452.
16 Wiffen PJ, McQuay HJ, Moore RA. Carbamazepine for acute and chronic pain. Cochrane Database Syst Rev 2005:CD005451.

High-Tone External Muscle Stimulation in End-Stage Renal Disease: Effects on Symptomatic Diabetic and Uremic Peripheral Neuropathy

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Objective and Design: Pain and peripheral neuropathy are frequent complications of end-stage renal disease (ESRD). Because drug treatment is associated with numerous side effects and is largely ineffective in many maintenance hemodialysis (MHD) patients, nonpharmacologic strategies such as electrotherapy are a potential recourse. Among various forms of electrostimulation, high-tone external muscle stimulation (HTEMS) is a promising alternative treatment for symptomatic diabetic peripheral polyneuropathy (PPN), as demonstrated in a short-term study. Based on these novel findings, we performed a prospective, nonrandomized, pilot trial in MHD patients to determine (1) whether HTEMS is also effective in treating diabetic PPN in the uremic state, and (2) whether uremic PPN is similarly modulated.

Patients and Interventions: In total, 40 MHD patients diagnosed with symptomatic PPN (25 with diabetic and 15 with uremic PPN) were enrolled. Both lower extremities were treated intradialytically with HTEMS for 1 hour, three times a week. Initially, a subgroup of 12 patients was followed for 4 weeks, and a further 28 patients for 12 weeks. The patients' degree of neuropathy was graded at baseline before HTEMS and after 1 and 3 months, respectively. Five neuropathic symptoms (tingling, burning, pain, numbness, and numbness in painful areas) as well as sleep disturbances were measured, using the 10-point Neuropathic Pain Scale of Galer and Jensen (*Neurology* 48:332-338, 1997). A positive response was defined as the improvement of one symptom or more, by at least 3 points. Other parameters included blood pressure, heart rate, dry body weight, and a routine laboratory investigation.

Results: The HTEMS led to a significant improvement in all five neuropathic symptoms, and to a significant reduction in sleep disturbances for both diabetic and uremic PPN. The response was independent of the patient's age, with a responder rate of 73%. The improvement of neuropathy was time-dependent, with the best results achieved after 3 months of treatment. The HTEMS was well-tolerated by nearly all patients.

Conclusions: This pilot study shows for the first time that HTEMS can ameliorate the discomfort and pain associated with both diabetic and uremic PPN in MHD patients, and could be a valuable supplement in the treatment of pain and neuropathic discomfort in patients who do not respond to, or are unable to participate in, exercise programs during hemodialysis treatment.

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PAIN IS A FREQUENT COMPLICATION of end-stage renal disease (ESRD), and has a profoundly negative impact on patients' quality of life. In particular, in peripheral diabetic neuropathy, loss of sensation is a forerunner of neuropathic ulcers and the leading cause of amputation.[1] According to a prospective Canadian study of maintenance hemodialysis (MHD) patients, pain was prevalent in >50% of patients, of whom 83% reported a pain intensity ranging from moderate to severe.[2] Most patients suffered from pain brought on by musculoskeletal disorders. The prevalence of pain because of peripheral

polyneuropathy (PPN) and peripheral vascular disease averaged 13% and 10%, respectively.[2] Moreover, the Pain Management Index was negative in 75% of patients, indicating the inefficacy of many analgetic drug therapies (anticonvulsants, tricyclic antidepressants, and opioids), which are associated with a multitude of side effects, such as sedation, nausea, and dry mouth.[2]

With regard to manifesting fewer side effects, nonpharmacologic strategies are of growing importance. Unfortunately, relaxation techniques, hypnosis, and meditation are of limited value. There is evidence that aerobic exercise training can modify the natural course of diabetic PPN, or even prevent its onset.[3] However, physical exercise (though strongly recommended) is difficult to realize for many (often immobile) MHD patients, who have a low physical functional capacity as a consequence of their multiple comorbidities.

In the management of pain, various forms of electro-medical therapy figure prominently. Most commonly used are transcutaneous electrical nerve stimulation (TENS)[4] and percutaneous electrical nerve stimulation (PENS), which combines TENS and acupuncture.[5] Both treatments were shown to ameliorate painful diabetic neuropathy. Another very effective therapy in patients with painful diabetic PPN is spinal-cord stimulation (SCS), which simultaneously improves exercise tolerance.[6] Spinal-cord stimulation also proved successful in patients with intractable angina pectoris, in whom angioplasty or coronary bypass graft surgery could not be performed.[7,8] This form of electrotherapy was also recommended for pain because of lower-limb ischemia in subjects unsuitable for vascular reconstruction.[9] Recently the effect of SCS was studied in eight MHD patients. Within a treatment period of 6 to 12 months, SCS dramatically lowered pain, improved patient quality of life, and appeared to delay ischemic skin lesions and amputation in patients at Leriche-Fontaine stage 2 or 3.[10] However, SCS therapy may be associated with severe complications, such as life-threatening infections (of 3% to 5% of SCS-treated patients).[11]

Within the new generation of electrotherapy techniques is the so-called "high-tone external muscle stimulation" (HTEMS). Whereas classical electrotherapy uses fixed carrier frequencies of typically 4000 Hz, the frequencies of HTEMS are continuously scanned from 4096 to 31,768 Hz, allowing for a much higher power of up to 5000 mW to be introduced to the muscles treated. In a short-term (3 consecutive days), comparative investigation in subjects with symptomatic diabetic PPN, HTEMS alleviated discomfort and pain more effectively than did TENS. Moreover, this kind of therapy exhibited no harmful side effects.[12]

Based on these novel findings, our objective was to determine (1) whether HTEMS is likewise effective in diabetic ESRD patients with symptomatic PPN, and (2) whether the uremic PPN is similarly modulated. Subsequently, a multicenter pilot study was conducted in MHD patients. The results indicated a significant improvement of pain and discomfort, in symptomatic uremic and diabetic PPN.

Methods

A prospective, nonrandomized, clinical pilot study was performed in MHD patients (with moderate discomfort or pain for at least 3 months) from five dialysis centers in Germany (KfH-Kidney Centers in Aschaffenburg, Frankfurt, Würzburg, the St. Marien Hospital in Duisburg) and Italy (Ospedale A. Landolfi, Solofra). In total, 40 patients, aged between 50 and 93 years (71.8 SD $\pm$ 11.2 years), and who had received hemodialysis for a minimum of 6 months, were enrolled. Patients receiving MHD and with complicating peripheral vascular disease were included in the investigation. Analgetic drugs were permitted, but were reduced to an as-needed basis.

We excluded patients with a pacemaker, a recent myocardial infarction, severe congestive heart failure, central neurologic disorders (e.g., Parkinson's disease, epilepsy, or multiple sclerosis), psychiatric disorders, and bacterial infections. The baseline characteristics of patients are summarized in Table 1.

The diagnosis of symptomatic PPN was based on the medical history, a neurologic investigation, and the presence of the following neuropathic symptoms: tingling, burning, pain, numbness, and numbness in painful areas, as well as sleep disorders.

External muscle stimulation was performed with a HiTop 184 appliance (GBO Medizintechnik, Rimbach, Germany), which is a nonportable 230-V power-supply device.[12] For HTEMS therapy, the electrodes were placed on the femoral muscles, and in some cases on the calves as well (Fig. 1). The intensity of electrical stimulation

Table 1. Characteristics of Forty Maintenance Hemodialysis Patients Who Participated in This Study

Patient Characteristics	All Patients (N = 40)	Diabetic PPN (n = 25)	Uremic PPN (n = 15)
Age (y) (mean ± SD)	71.8 ± 11.2	71.2 ± 11.4	72.8 ± 11.1
Age range (y)	50–93	51–93	50–87
Sex (men/women)	25/15	16/9	9/6
Renal diagnoses			
Diabetic nephropathy	24	24	
Chronic glomerulonephritis	6		6
Ischemic renal failure	5		5
Pyelonephritis	1		1
Wegener's Granulomatosis	2		2
Polycystic kidney disease	2	1	1
Peripheral arterial disease	8	7	1

PPN, peripheral polyneuropathy.

was adjusted to suit the comfort level of each individual patient without producing discomfort or pain. All subjects were treated for 1 hour during the hemodialysis session, three times weekly. Initially, 12 patients were followed for 1 month, and a further 28 patients were followed for a treatment period of 3 months.

The primary outcome measure was the potential modulation of intensity of neuropathic symptoms. For purposes of grading these symptoms, patients were requested to record their baseline levels of pain and discomfort, using a visual 10-point analogue scale, ranging from 0 ("no symptoms") to 10 ("worst ever felt").[13] Improvement was defined as a decline of ≥3 points of at least one symptom.

Other recorded parameters included systolic and diastolic blood pressure, heart rate, and dry body weight. Further, a routine laboratory investigation was performed, including measurements of albumin, C-reactive protein, and Hemoglobin A1c (HbA1c) in diabetics.

All data are expressed as mean ± SD. Changes in the investigated parameters were calculated by paired Student *t* test (two-tailed), with $P < .05$ considered statistically significant. All analyses were performed using Microsoft Excel (Redmond, WA).

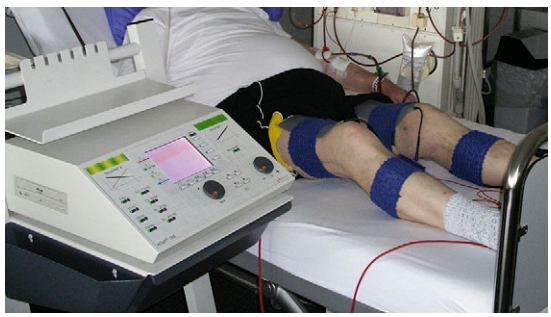

Figure 1. High-tone external muscle stimulation of thighs and calves in a maintenance hemodialysis patient with the HiTop 184 device (GBO Medizintechnik, Rimbach, Germany).

Results

In our 40 MHD patients, the neuropathic symptoms of pain and discomfort exhibited significant improvements in terms of tingling, burning, pain, numbness, and numbness in painful areas ($P < .005$). Even the sleep disorders induced by neuropathy were significantly improved during long-term HTEMS therapy ($P < .005$) (Fig. 2). The amelioration of symptoms of peripheral neuropathy was observed after only 4 weeks of HTEMS treatment in a subgroup of 12 patients. Concerning the underlining neuropathy, the response was significant in both uremic and diabetic polyneuropathy, with the exception of the neuropathic symptom "numbness in painful areas." In that case, the improvement in patients with uremic polyneuropathy was not statistically significant. The response was independent of patients' age or sex.

When defining a positive response as the improvement of one symptom or more by at least 3 points, the response rate for all 40 MHD patients averaged 73% (29 out of 40). In the subgroup of patients with uremic PPN, the response rate was higher compared with their counterparts with diabetic PPN (11 out of 15, or 80%, versus 18 out of 25, or 72%).

When comparing the improvement of neuropathic symptoms in the initial subgroup of 12 patients treated with HTEMS for 4 weeks with the 28 patients treated for 12 weeks, the difference

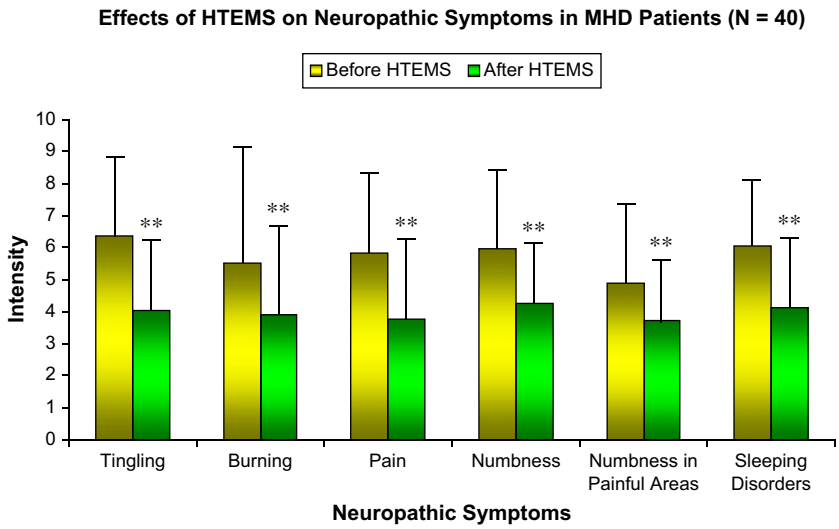

Figure 2. Effects of high-tone external muscle stimulation (HTEMS) on five neuropathic symptoms and on sleeping disorders in maintenance hemodialysis patients. (**$P < .005$).

in response rate was considerable, at 58% (7 out of 12) versus 79% (22 out of 28), respectively. The response rate was clearly dependent on the duration of HTEMS treatment.

The HTEMS was well-tolerated by all subjects in the study, apart from one patient who reported enhanced nervousness and sleeplessness after only three sessions of treatment. She was subsequently excluded from the investigation. After 4 to 6 weeks, some patients reported an increase in overall well-being, accompanied by a rise in muscle strength.

During the treatment period, HTEMS had no significant effect on systolic and diastolic blood pressure or on heart rate. Similarly, there were no significant changes in dry body weight. Concerning blood chemistry, the albumin concentration was followed in 26 MHD patients. Whereas there was no significant change within the first 4 weeks of HTEMS, after 12 weeks of treatment, a clear trend toward a higher albumin concentration was observed. There were no significant changes in C-reactive protein and HbA1C in the diabetic MHD patients.

Discussion

In our prospective, nonrandomized pilot study of MHD patients, we found significant improvement in the neuropathic symptoms of tingling, burning, pain, numbness, and numbness in painful areas. The response was comparable between patients with diabetic PPN and those with uremic PPN. This implies that HTEMS alleviates neuropathic symptoms of different pathogeneses. In addition, patients' quality of sleep was significantly improved, in terms of less frequent nighttime waking on account of pain and discomfort. The treatment effects were clearly dependent on the duration of HTEMS therapy, with the best results observed after >4 weeks.

Our data correspond with, and extend, the earlier investigation of short-term (3 consecutive days) HTEMS treatment.[12] Similarly positive observations were made by Humpert et al.[17] during an 8-week treatment period in 27 patients with painful diabetic neuropathy.

To date, the mechanisms underlying the improvement in neuropathic symptoms after electrotherapy are not well-understood. In various investigations, improved microcirculation after electrotherapy was shown.[17–19] This effect is particularly pronounced when using epidural spinal-cord electrical stimulation in patients with severe limb ischemia.[9] Electrical stimulation was also shown to improve wound-healing in patients with diabetic ulcers.[20] Enhanced microcirculation, as induced by electrical stimulation, could be of therapeutic value with regard to the lowered microcirculation in the peripheral nerves of patients with diabetic neuropathy.

Another possible underlying mechanism of electrotherapy is the activation of dorsal columns. Here, pain input is interrupted via inhibition of

the C fibers.[14] Recently, high-frequency TENS was shown to decrease human motor cortex excitability.[15] Further, it decreased levels of the excitatory amino acids, glutamate and aspartate, in the dorsal horn.[16]

Of particular interest are the metabolic effects of electrotherapy, which was shown to increase muscle oxidative capacity.[21] Electrical stimulation of the lower extremities of healthy humans was shown to enhance energy consumption, carbohydrate oxidation, and whole-body glucose uptake during a euglycemic clamp.[22] Compared with voluntary cycling exercise at an identical intensity, electrostimulation resulted in a higher respiratory gas exchange ratio, indicating higher carbohydrate oxidation. Furthermore, the enhanced glucose removal was not limited to the exercise period, but persisted after the exercise period for at least 90 minutes, in contrast to voluntary exercise.[23] In paraplegic patients, 8 weeks of treatment (3 hours/week) enhanced the glucose transporters 1 and 4, oxidative capacity, and insulin sensitivity.[24]

In line with these observations of enhanced glucose metabolism, HTEMS (1 hour/day) induced a significant improvement in HbA1c (–0.6%) over a treatment period of 6 weeks, associated with a decline in body weight (–1.4 kg).[25] After the discontinuation of therapy, a renewed deterioration of these parameters occurred. In contrast to these findings, we did not observe a significant change in HbA1c in our diabetic MHD patients, which may be a consequence of the lower treatment frequency of HTEMS therapy in our investigation (1 hour, 3 times/week). In fact, in two other patients treated daily with HTEMS (data not shown), an improvement in HbA1c and overweight was observed.

Aerobic and resistance exercise are generally recommended in MHD, particularly to improve the prevalent sarcopenia and physical fitness of these patients.[26] In principle, similar positive effects could be achieved by HTEMS in immobile patients with low physical functional capacity, making electrotherapy a viable, intermediate treatment strategy.

Conclusion

According to our data, long-term treatment with HTEMS in MHD patients can lead to significant improvement of neuropathic symptoms. Many patients also reported a subjective improvement of their general well-being, with greater muscle strength. The greatest shortcoming of our study was the lack of a control group, insofar as no placebo intervention could be implemented. High-tone external muscle stimulation should be considered for patients with symptomatic peripheral neuropathy who do not respond to, or are unable to participate in, exercise programs during hemodialysis treatment.

References

1. Davies M, Brophy S, Williams R, Taylor A: The prevalence, severity, and impact of painful diabetic peripheral neuropathy in type 2 diabetes. Diabetes Care 29:1518-1522, 2006

2. Davison SN: Pain in hemodialysis patients: prevalence, cause, severity, and management. Am J Kidney Dis 42: 1239-1247, 2003

3. Balducci S, Iacobellis G, Parisi L, et al: Exercise training can modify the natural history of diabetic peripheral neuropathy. J Diabetes Complications 20:216-223, 2006

4. Kumar D, Marshall HJ: Diabetic peripheral neuropathy: amelioration of pain with transcutaneous electrostimulation. Diabetes Care 20:1702-1705, 1997

5. Hamza MA, White PF, Craig WF, et al: Percutaneous electrical nerve stimulation: a novel analgesic therapy for diabetic neuropathic pain. Diabetes Care 23:365-370, 2000

6. Tesfaye S, Watt J, Benbow SJ, et al: Electrical spinal-cord stimulation for painful diabetic peripheral neuropathy. Lancet 348:1698-1701, 1996

7. Mannheimer C, Augustinsson LE, Carlsson CA, et al: Epidural spinal electrical stimulation in severe angina pectoris. Br Heart J 59:56-61, 1988

8. Mannheimer C, Eliasson T, Augustinsson LE, et al: Electrical stimulation versus coronary artery bypass surgery in severe angina pectoris: the ESBY Study. Circulation 97:1157-1163, 1998

9. Jacobs MJ, Jorning PJ, Joshi SR, et al: Epidural spinal cord electrical stimulation improves microvascular blood flow in severe limb ischemia. Ann Surg 207:179-183, 1988

10. Brümmer U, Condini V, Cappelli P, et al: Spinal cord stimulation in hemodialysis patients with critical lower-limb ischemia. Am J Kidney Dis 47:842-847, 2006

11. Torrens JK, Stanley PJ, Ragunathan PL, Bush DJ: Risk of infection with electrical spinal-cord stimulation. Lancet 349: 729, 1997

12. Reichstein L, Labrenz S, Ziegler D, Martin S: Effective treatment of symptomatic diabetic polyneuropathy by high-frequency external muscle stimulation. Diabetologia 48:824-828, 2005

13. Galer BS, Jensen MP: Development and preliminary validation of a pain measure specific to neuropathic pain: the neuropathic pain scale. Neurology 48:332-338, 1997

14. Watkins ES, Koeze TH: Spinal cord stimulation and pain relief. Br Med J [Clin Res] 307:462, 1993

15. Mima T, Oga T, Rothwell J, et al: Short-term high-frequency transcutaneous electrical nerve stimulation decreases human motor cortex excitability. Neurosci Lett 355:85-88, 2004

16. Lundeberg T, Kjartansson J, Samuelsson U: Effect of electrical nerve stimulation on healing of ischaemic skin flaps. Lancet 2:712-714, 1988

17. Humpert PM, Rudofsky G, Morcos M, et al: Hochfrequente Muskelstimulation zur Behandlung schmerzhafter Neuropathie bei Typ 2 Diabetikern verbessert die mikrovaskuläre Endothelzellfunktion. Diab Stoffw 128(Suppl 1):S63, 2006

18. Tesfaye S, Harris ND, Wilson RM, Ward JD: Exercise-induced conduction velocity increment: a marker of impaired peripheral nerve blood flow in diabetic neuropathy. Diabetologia 35:155-159, 1992

19. Tesfaye S, Harris ND, Jakubowski JJ, et al: Impaired blood flow and arterio-venous shunting in human diabetic neuropathy: a novel technique of nerve photography and fluorescein angiography. Diabetologia 36:1266-1274, 1993

20. Baker LL, Chambers R, DeMuth SK, Villar F: Effects of electrical stimulation on wound healing in patients with diabetic ulcers. Diabetes Care 20:405-412, 1997

21. Martin TP, Stein RB, Hoeppner PH, Reid DC: Influence of electrical stimulation on the morphological and metabolic properties of paralyzed muscle. J Appl Physiol 72:1401-1406, 1992

22. Hamada T, Sesaki H, Hayashi T, et al: Enhancement of whole body glucose uptake during and after human skeletal muscle low-frequency electrical stimulation. J App Physiol 94: 2107-2112, 2003

23. Hamada T, Hayashi T, Kimura T, et al: Electrical stimulation of human lower extremities enhances energy consumption, carbohydrate oxidation, and whole body glucose uptake. J Appl Physiol 96:911-916, 2004

24. Chilibeck PD, Bell G, Jeon J, et al: Functional electrical stimulation exercise increases GLUT-1 and GLUT-4 in paralyzed skeletal muscle. Metabolism 48:1409-1413, 1999

25. Rose B, Lankisch M, Herder C, et al: Externe Muskelstimulation beeinflusst die Diabeteseinstellung: eine neuartige Therapieoption für Typ 2 Diabetes. Diabetologie und Stoffwechsel 2(Suppl 1):S107, 2007

26. Johansen KL: Exercise in the end-stage renal disease population. J Am Soc Nephrol 18:1845-1854, 2007

High-Tone External Muscle Stimulation in End-Stage Renal Disease: Effects on Symptomatic Diabetic and Uremic Peripheral Polyneuropathy

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Introduction:

- Pain is a frequent complication of end-stage renal disease.
- Drug treatment: numerous side-effects, largely ineffective in many maintenance hemodialysis (MHD) patients.
- Non-pharmacological strategies, e.g. electrotherapy
- ➔High-Tone External Muscle Stimulation (HTEMS): a promising treatment of symptomatic diabetic peripheral polyneuropathy (PPN).

Objectives:

In a prospective, non-randomized, pilot trial in MHD patients we aimed to determine whether HTEMS is effective in symptomatic diabetic and uremic PPN.

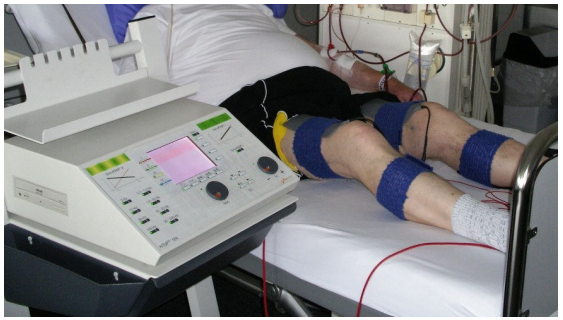

Figure 1: High-tone external muscle stimulation of thighs and calves in a maintenance hemodialysis (MHD) patient with the HiTop 184 device (gbo Medizintechnik AG, Rimbach, Germany).

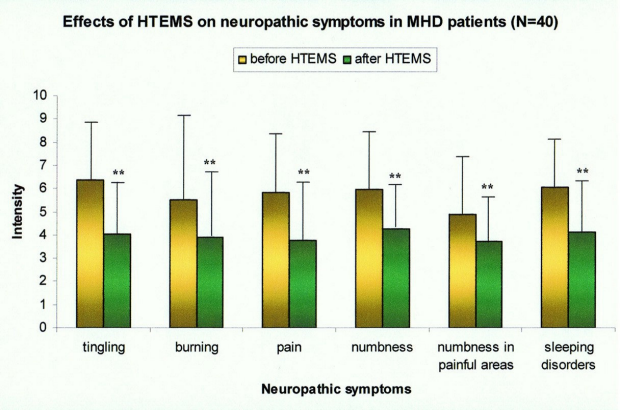

Figure 2: Effects of high-tone external muscle stimulation (HTEMS) on 5 neuropathic symptoms and on sleeping disorders in maintenance hemodialysis patients before and after HTEMS, (**: p<0.005)

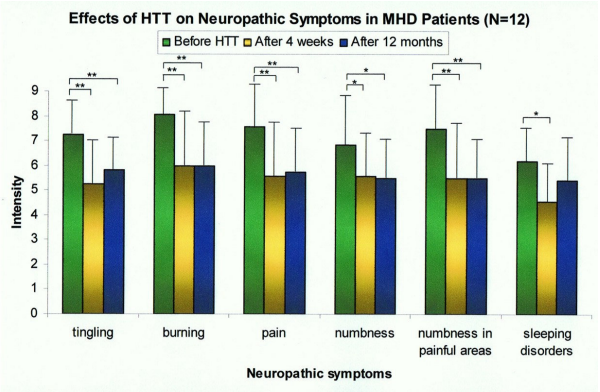

Figure 3: Effects of high-tone external muscle stimulation (HTEMS) on 5 neuropathic symptoms and on sleeping disorders in maintenance hemodialysis patients after 4 weeks and 1 year, respectively (*: p<0.05, **: p<0.005)

Design and Subjects:

- N=40 MHD patients with symptomatic PPN, diabetic (n=25) and uremic (n=15) were intradialytically treated with HTEMS for 1 hour, thrice weekly.
- Degree of neuropathy was graded at baseline before and after continued HTEMS with the 10-point Neuropathic Pain Scale of Galer & Jensen (1997): 5 neuropathic symptoms (*tingling*, *burning*, *pain*, *numbness*, *numbness in painful areas*). A positive response was defined as the improvement of one symptom or more, by at least three points.
- Measurement of *sleeping disturbances.*

Results:

- A significant improvement in all 5 neuropathic symptoms, as well as in sleeping disorders (Fig. 2)
- Responder rate: 73%.
- The beneficial neuropathic effects were still evident in 12 MHD patients on HTEMS after 1 year (Fig. 3).
- HTEMS was well-tolerated by nearly all patients.

Conclusion:

- **In MHD patients, HTEMS ameliorates the discomfort and pain associated with diabetic and uremic PPN.**
- **HTEMS is a valuable supplement in the treatment of pain and neuropathic discomfort.**

Neuro-muscular electro-stimulation: Outlooks for patients with renal disease

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It is our pleasure to provide the readers of Clinical Nephrology the lecture contributions of the International Workshop on Electrical Muscle Stimulation: Perspectives in Patients with Renal Disease, which took place on September 9, 2010 in Taormina, Sicily, Italy. The symposium was organized to present and discuss the results of a new form of electrotherapy, the so-called high-tone external muscle stimulation (HTEMS). The value of this treatment lies in its marked analgesic effects in various pain conditions, in particular in peripheral neuropathy. For dialysis patients, its application is especially convenient, as it can be administered during the dialysis session and without any relevant side effects.

Aside from its pain-relieving properties, HTEMS may beneficially modulate the course of acute renal failure, by influencing protein catabolism and renal function. The first observations are a challenge to perform further, in-depth investigations.

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High-tone external muscle stimulation in patients with acute kidney injury (AKI): beneficial effects on NO metabolism, asymmetric dimethylarginine, and endothelin-1

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Key words
acute kidney injury – nitric oxide – ADMA – endothelin-1 (ET-1) – electrical muscle stimulation

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Abstract. Objectives: The aim of this study was to assess potential effects of high-tone external muscle stimulation (HTEMS) on parameters of endothelial dysfunction (ED) in patients with acute kidney injury (AKI). Background: The bad outcome of AKI patients is markedly influenced by ED, microinflammation, oxidative stress and protein hypercatabolism. Recently, we have shown that intradialytic application of HTMS was associated with a faster resolution of AKI. Here, we investigated in the same cohort of patients whether parameters of ED such as nitric oxide (NO), asymmetric-dimethylarginine (ADMA), and endothelin 1 (ET-1) are modulated by HTEMS as compared to non-HTEMS-treated AKI patients. Methods: In a post-hoc study we analyzed plasma samples of the 34 AKI patients stage 5, of whom 17 underwent intradialytic HTEMS treatment while the other 17 served as AKI dialysis controls. Measurements included plasma nitrate and nitrite (NOx), ADMA, ET-1 and were performed before and on days 3, 7, 14, 21, and 28 after start of daily dialysis. Additional 16 healthy volunteers served as controls. Results: Initially, in both AKI groups NOx levels were markedly lower and ADMA and ET-1 levels were higher compared to the healthy controls. After initiation of daily hemodialysis the HTEMS group showed a faster improvement of NOx and ET-1 (after 1 week) and ADMA levels (after 2 weeks) compared to the No-HTEMS group. After 2 weeks, all parameters of the HTEMS group were not different from healthy controls, while the No-HTEMSAKI group needed 3 – 4 weeks. Conclusion: Our findings suggest for the first time that in AKI patients, application of HTEMS is associated with a faster normalization of lowered NOx and elevated ADMA and ET-1 plasma levels. We hypothesize that the more rapid amelioration of these parameters in the HTEMS group contributed to the accelerated recovery of AKI. With regard to the small study groups with different causes of AKI, investigations in a greater number of AKI patients is required.

Introduction

Acute kidney injury (AKI) comprises a multifactorial and heterogeneous disorder and is associated with a high morbidity and mortality rate, especially in intensive care patients [1, 2]. The bad outcome in AKI is caused in particular by non-renal complications of heart, liver, lung, and brain and the still limited therapeutic options.

Up to now the pathogenesis of AKI is poorly understood. In ischemic AKI, reduction of renal blood flow, in particular of the outer medulla with subsequent hypoxia and activation of inflammatory pathways, seem of essential importance [3]. Recently, the key role of endothelial dysfunction and disturbed nitric oxide (NO) formation has been emphasized [4]. Lowered NO formation is associated with loss of vasorelaxation, enhanced adhesion of polymorphonuclear neutrophils (PMN), and impaired antithrombotic activity of the endothelium [5]. Relevant insults of the kidney are succeeded by release of numerous "danger signals" into the circulation. They include multiple cytokines and chemokins,

Table 1. Characteristics and medication of the patients with AKI treated during hemodialysis with or without HTEMS (mean ± SD).

	HTEMS n = 17	No HTEMS n = 17
1A: Characteristics of the patients		
Sex males	10	11
Age (years)	71.4 ± 11.2	69.6 ± 9.1
Body weight (kg)	65.1 ± 9.2	66.6 ± 0.3
Body mass index (kg/m^2)	25.1 ± 1.2	24.6 ± 1.5
History of hypertension	16	15
Diabetes mellitus (Type 2)	12	9
NYHA > 3	14	10
Systolic blood pressure mmHg)	102 ± 11	110 ± 12
Diastolic blood pressure (mmHg)	65 ± 16	61 ± 11
1B: Medications		
ACE inhibitors	11	10
Angiotensin II type 1 receptor blockers	12	13
Beta-receptor blockers	7	8
Calcium channel blockers	10	9
Vasodilating drugs	6	8
Furosemide	15	14
Hydrochlorothiazide	6	7

There were no significant differences of all parameters between the groups.

reactive oxygen species (ROS), the proinflammatory high mobility group box protein 1 (HMGB1), and various vasoconstrictors (endothelin-1 (ET-1), angiotensin II and catecholamines), which predispose to dysfunction of other organs ("distant organ effects of damaged kidney") [6]. Concomitant and/or preexisting diseases aggravate the inflammatory response and oxidative stress [6]. An important hallmark of AKI is the accelerated protein breakdown due to the acute disease process, immobilization, systemic inflammation, insulin resistance, metabolic acidosis, hormonal derangements, circulating proteases, and type of renal replacement therapy (RRT) [7].

In established AKI the kinds of pharmacotherapeutic possibilities are mostly confined to supportive treatment such as correction of blood pressure disturbances, hyper-/hypovolemia, electrolyte disturbances, metabolic acidosis, hyperglycemia, nutritional support, and RRT. Therefore, new therapeutic strategies are a challenge. One possibility could be an intervention at the muscular level. It is wellknown that aerobic and/or resistance exercise exerts anti-inflammatory and anti-oxidative effects [8]. It prevents or reduces muscle degradation, improves insulin sensitivity, and increases NO formation [9, 10]. For this reason, in maintenance dialysis patients aerobic and resistance exercise is strongly recommended with great success [11]. Unfortunately, in severely ill patients physical exercise is often limited or impossible. In this situation, an alternative could be neuromuscular electrical stimulation (NMES). This kind of therapy mimics various effects of voluntary exercise; it increases the oxidative capacity of muscles, the glucose disposal [12] and augments the microcirculation, even in distant vessels [13]. Sarcopenia was improved in patients with congestive heart failure [14] and end-stage renal disease [15]. In critically ill patients electrical muscle stimulation may preserve the muscle mass [16]. In particular, low frequency transcutaneous electrical stimulation (TENS) was effective [17], but also high frequency NMES of rat lower legs activated the Glut 4 transporter and increased muscle mass [18]. In obese diabetic patients, daily application of high-frequency external muscle stimulation, also called high-tone external muscle stimulation (HTEMS) ameliorated the HbA1c level and HOMA index [19], indicating an improved insulin sensitivity.

Recently, we could show in patients with AKI that intradialytic application of HTEMS to both lower legs was associated with a shortened recovery of AKI [20]. To elucidate the biological mechanisms behind this beneficial action, we assessed now in a post-hoc analysis the plasma samples of these AKI patients for parameters of endothelial dysfunction such as oxidation products of NO (nitrate and nitrite), asymmetric dimethylarginine (ADMA), and endothelin-1(ET-1). In particular, plasma nitrite concentration reflects the degree of endothelial dysfunction in humans [21].

Methods

Study population

This study is a post-hoc analysis of previous investigations [20]. As described, 34 consecutive oliguric patients with AKI were randomized into two groups of 17 patients each with or without HTEMS therapy during daily hemodialysis treatment. The characteristics and medications of the AKI patients are shown in Table 1 and the causes of AKI and the blood chemical parameters are presented in Table 2. Both study groups had almost

Table 2 . Causes of AKI and blood chemical values at admission.

	HTEMS	No HTEMS	p
2A: Causes of AKI			
Drugs			
Nonsteroidal anti-inflammatory drugs	3	5	
Aminoglycosides	2	3	
Allopurinol	2	1	
Acute heart failure	5	3	
Dehydration following gastroenteritis	2	2	
Acute hyperuricemic nephropathy	1	2	
Acute hepatorenal syndrome	2	1	
2B: Blood chemistry			
Plasma creatinine (mg/dL)	6.4 ± 1.3	6.2 ± 1.6	0.692
Urea (mg/dL)	300 ± 78	290 ± 84	0.72
Urea/creatinine ratio	30 ± 13	29 ± 12	0.817
Hemoglobin (g/dL)	9.8 ± 0.5	10 ± 0.6	0.115
Albumin (g/dL)	3.0 ± 1.0	2.9 ± 0.9	0.319
C-reactive protein (mg/l)	8.5 ± 0.9	6.6 ± 1.3*	0.05
Bicarbonate (mEq/L)	15.2 ± 2.6	16.0 ± 2.5	0.367

*There were no significant differences of blood chemical parameters between the groups except for C-reactive protein ($p < 0.05$).

comparable causes of AKI (mostly toxic or ischemic), a similar pharmacotherapy and nearly identical baseline biochemical parameters [20].

A group of healthy 16 subjects, 9 males and 7 females, age 57 ± 14 years, served as controls for NOx, ADMA, and ET-1.

Medical treatment of the AKI patients

Antihypertensive treatment was reduced and combination of ACE inhibitors and AT1 receptor blocker excluded. Dehydration of the AKI patients was treated by adequate fluid substitution. To promote diuresis and reduce intra-renal oxygen consumption, intravenous furosemide (0.75 ± 0.3 mg/kg/bw/d) was administered. In the case of metabolic acidosis, sodium bicarbonate was given to achieve a plasma bicarbonate level of more than 22 mEq/L.

Dialysis modalities

All AKI patients were oliguric (urine output < 400 mL/24 h) and were treated with daily hemodialysis. When the diuresis exceeded 700 mL/24 h, dialysis was performed on alternate days. Renal replacement treatment was stopped when renal function returned to stage 4 of kidney disease (eGFR: 15 – 29 mL/min). Hemodialysis sessions were performed daily over 4 hours with Hemo-Flow using polymethylacrylamide membranes, surface area 1.4 – 2.0 m^2. The blood flow averaged 250 mL/min. The dialysate fluid had the following electrolyte concentration: sodium 140 – 145 mmol/L, potassium 2 – 4 mmol/L according to plasma potassium, calcium 1.5 mmol/L and bicarbonate 28 – 30 mEq/L [20].

Intervention by HTEMS application during daily hemodialysis

HTEMS was performed with a HiToP 184 appliance (gbo Medizintechnik AG, Rimbach, Germany), which is a 230 V power supply device [22]. In this new electro-medical approach, a continuous scan of the carrier frequency between 4,100 and 33,000 Hz is associated with frequency-dependent modulation of the amplitude. The electrodes were placed around the femoral muscles and in some cases on the calves as well. Intensity of electrical stimulation was adjusted to suit the comfort level of each patient without produc-

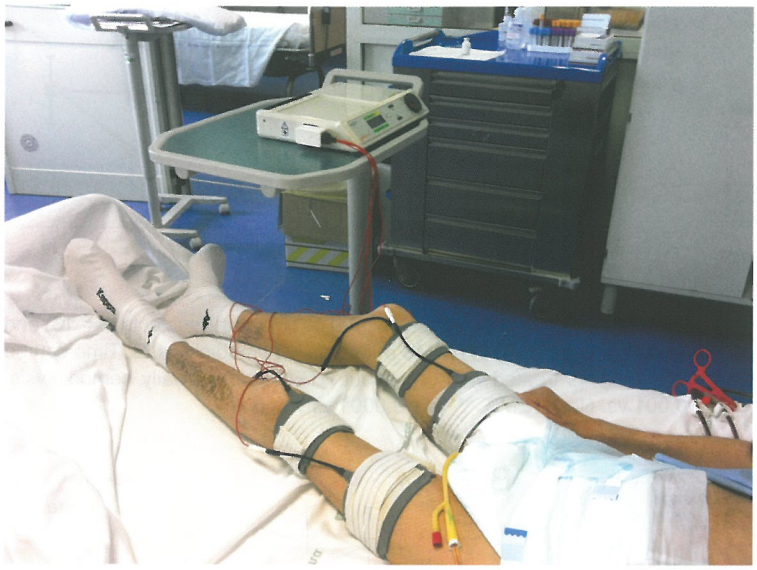

Figure 1. High-tone electrical muscle stimulation of thighs with a HiTop184 device (gbo Medizintechnik AG).

ing pain (Figure 1). HTEMS was performed for 1 hour during each dialysis treatment. This therapy is widely used in pain treatment of patients with diabetic and/or uremic polyneuropathy and is well tolerated [22, 23, 24].

HTEMS was stopped when dialysis was no longer necessary. Patients in both groups were bedridden during dialysis treatment time. Blood samples were taken daily to verify the clinical condition of the patients and the levels of nitrogen catabolites. We hypothesized that earlier recovery of AKI in subjects with HTEMS therapy may be mediated in part via vasodilatory mechanisms. Therefore we measured NOx, ADMA, and ET-1.

Plasma analysis

We measured from the stocked plasma samples nitrite/nitrate, ET-1 and ADMA concentrations.

Measurement of nitrite/nitrate concentrations

Plasma nitrite/nitrate (NOx) concentration is a surrogate marker of NO generation. For its measurement, plasma samples were incubated with nitrate reductase (0.1 U/mL), NADPH (1 mm) and FAD (50 µm) at 37 °C [25]. After 15 minutes, samples were incubated with LDH (100 U/mL) and sodium pyruvate (10 mm) for 5 minutes. The total NOx concentration was measured by the Griess reaction after adding 100 µL of Griess reagent (0.1% naphthylethylenediamide dihydrochloride in H_2O and 1% sulfanilamide in 5% conc. H_2PO_4; vol. 1 : 1) to 100 µL of samples, each in triplicate. The optical density at 550 nm (OD550) was measured at 540 nm in a microplate reader Titertek (Dasit, Cornaredo, Milan, Italy). Total NOx concentrations (µm) were calculated from a standard curve of sodium nitrate.

Measurement of ADMA concentration

Plasma ADMA levels were analyzed with a commercially available enzyme-linked immunosorbent assay (ELISA) according to manufacturer's instruction (DLD Diagnostika, Hamburg, Germany). ADMA levels are expressed as mean ± SD in µmol/L.

Measurement of ET-1 concentration

ET-1 plasma concentration was analyzed by a commercial ELISA kit for human ET-1 according manufacturer's instruction (R&D Systems, Minneapolis, MN, USA). ET-1 levels are expressed as mean ± SD in pg/mL.

Statistics

All values are reported as means ± SD unless otherwise specified. Analysis of variance (ANOVA) was used to compare the two groups. Statistical significance was defined as $p < 0.05$.

Results

There was no difference between the two AKI groups regarding age, systolic and diastolic blood pressure, plasma creatinine, urea, hemoglobin and albumin (Tables 1 and 2). Only C-reactive protein (CRP) was higher in the HTEMS than in the No- HTEMS patients ($p < 0.001$) [20].

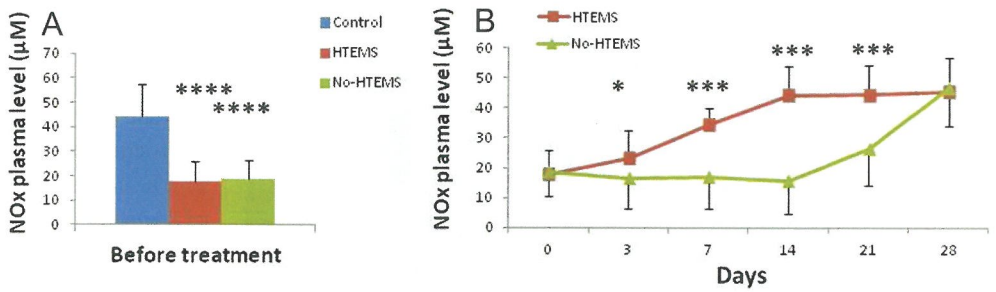

Figure 2. A: Baseline plasma values of NOx (sum of nitrate and nitrite) of 16 healthy subjects (n = 16) and of 34 patients with AKI treated with (17) or without (17) HTEMS during daily hemodialysis, B: Time course of plasma NOx concentration in the patients treated with or without HTEMS during daily hemodialysis. *p < 0.05 vs. No-HTEMS; ***p < 0.001 vs. No-HTEMS, ****p < 0.0001 vs. control.

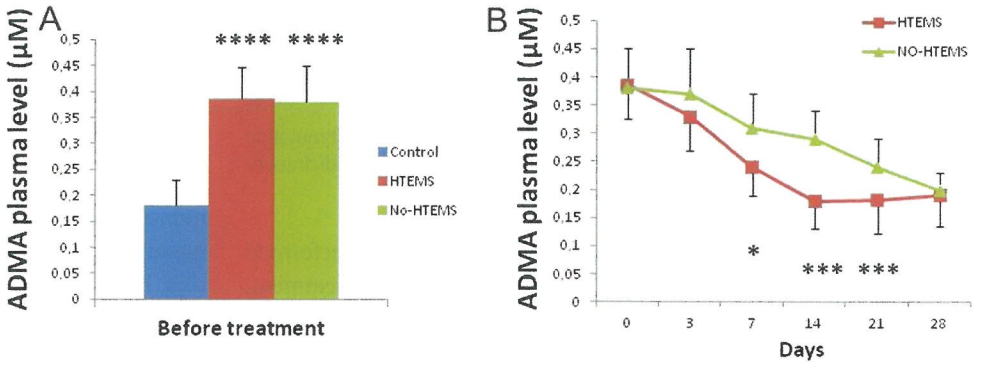

Figure 3. A: Baseline values of plasma asymmetric dimethylarginine (ADMA) concentrations of 16 healthy subjects (n = 16) and of 34 patients with AKI treated with (17) or without (17) HTEMS during daily hemodialysis; B: Time course of ADMA levels in the patients treated with and without HTEMS. *p < 0.05 vs. No-HTEMS; ***p < 0.001 vs. No-HTEMS; ****p < 0.0001 vs. control.

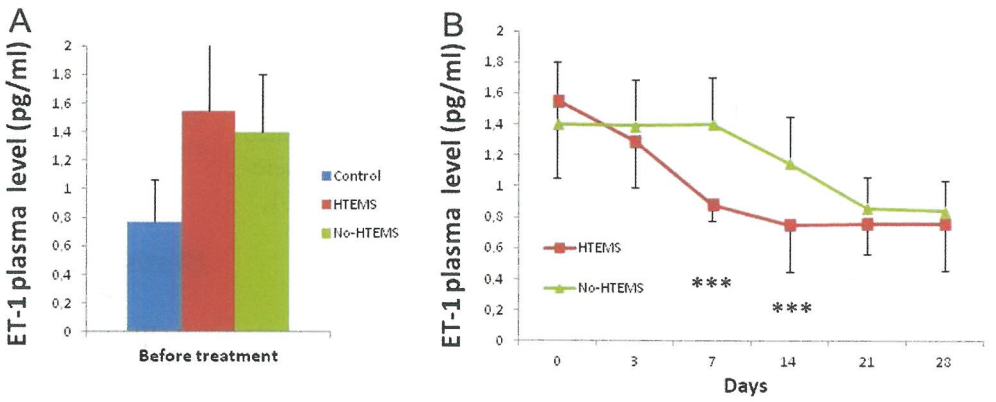

Figure 4. A: Baseline values of plasma endothelin-1 (ET-1) concentrations of 16 healthy subjects (n = 16) and of 34 patients with AKI treated with (17) or without (17) HTEMS during daily hemodialysis; B: time course of the ET-1 levels in patients treated with and without HTEMS. *p < 0.05 vs. No-HTEMS; ***p < 0.001 vs. No-HTEMS, ****p < 0.0001 vs. control.

The baseline values of NOx of both AKI groups were markedly lower than that of the controls ($p < 0.0001$) (Figure 2A). In the following days its concentration increased in the HTEMS group faster than in the No-HTEMS group. At the 7th day of AKI, the NOx values were statistically not different from the healthy controls, while the No-HTEMS patients normalized their NOx levels at the 21st day of AKI (Figure 2B).

The baseline levels of ADMA were markedly enhanced in both AKI groups as compared to the healthy controls ($p < 0.0001$) (Figure 3A). During daily hemodialysis, the ADMA

levels in the intervention group were significantly lower at the 7th day than in the untreated dialysis group ($p < 0.05$); after 14 days, the significance reached $p < 0.001$ (Figure 3B).

Baseline values of ET-1 levels were markedly elevated in both AKI groups as compared to the healthy controls ($p < 0.0001$) (Figure 4A). After 7 days of treatment the ET-1 values were significantly lower in the intervention group ($p < 0.001$) (Figure 4B).

The reduction of ET-1 values below 1 pg/mL occurred earlier in HTEMS group than in the No-HTEMS group (6.94 ± 0.97 vs. 20.94 ± 1.95 days, respectively; $p = 0.01$). The reduction of Cr < 50% occurred after ET-1 reduction < 1 pg/mL similarly in both groups (HTEMS 1.59 ± 0.62 days and No-HTEMS 1.82 ± 0.64 days, p = NS).

Discussion

In our previous study, we have reported that in AKI patients the intradialytic application of HTEMS shortened the clinical course [20]. As compared to untreated patients, the HTEMS group had a significantly reduced duration of oliguria, faster reduction of creatinine, less need of dialysis treatment and hospitalization time [20]. In the present study, we tried to elucidate the potential mechanisms involved in the improved clinical course. We could demonstrate that in the HTEMS-treated patients, the disturbed plasma levels of NOx, ADMA, and ET-1 were normalized faster than in the No-HTEMS group. These findings suggest that the shortened course of AKI might be a consequence of an improved endothelial dysfunction.

Parameters of endothelial dysfunction before start of hemodialysis therapy

In our study, the baseline values of NOx were lowered by 60% as compared to healthy controls. This decline was more pronounced than the plasma NOx level in AKI patients after cadaveric kidney transplantation, which was only 22% less (n.s.) than their healthy controls [26]. The stronger decline of NOx in our AKI patients may be a consequence of a generalized endothelial dysfunction and unlike the kidney transplantation patients not limited to the vascular bed of the transplanted organ. A decreased NO formation may occur even in early stages of AKI as shown in pediatric patients by determination of urinary nitrate excretion [27].

Decreased NO formation seems to be of fundamental importance in the pathogenesis of AKI, since NO is involved in the regulation of renal blood flow, glomerular and medullary hemodynamics, tubulo-glomerular feedback, renin secretion, and extracellular fluid volume homeostasis [28, 29]. Ischemic insults of the kidney inhibit eNOS and nNOS activities followed by diminished NO formation. Subsequent impairment of GFR and peritubular microcirculation contributes to hypoxia, oxidative stress, and inflammation [5, 30]. The importance of decreased NO production in AKI is underlined by the observation that topical sodium nitrite administration in rat ischemic kidney injury protected the damaged organ via an enhanced NO generation [29].

Reduced NOx levels of our patients may be a consequence of various factors such as enhanced formation of reactive oxygen species (ROS) [30], uremic retention products (toxic guanidine compounds [31], advanced glycation end products [32]) and a reduced dietary intake of nitrate [33]. Moreover the marked rise of the basal plasma ADMA levels (211%) of our patients can contribute to lowered NOx level by inhibition of nitric oxide synthase activity [34, 35]. ADMA limits the substrate (L-arginine) availability for NO formation (due to lowered renal L-arginine synthesis) and/or decreased L-arginine uptake into endothelial cells [36]. ADMA also directly interfere with eNOS and nNOS activities.

The baseline ET-1 plasma levels of the AKI patients were elevated (190%) as compared to the healthy controls. Several studies demonstrated that ET-1 (mostly secreted by endothelial cells) is an important pathogenetic factor of ischemia-induced AKI [37]. The toxicity is a consequence of its strong vasoconstrictive, proinflammatory, and mitogenic properties [37, 38]. In the kidney, ET-1 (as well as angiotensin II) causes a decrease of renal blood flow and GFR with a diminished circulation in the outer medullary descending vasa recta [37]. In mice experiments, deletion of ET-1 from endothelial cells attenuated the ischemia-induced kidney injury [38].

Effects of daily intradialytic HTEMS application on parameters of endothelial dysfunction

Daily HTEMS treatment during hemodialysis was followed by a faster improvement of the disturbed parameters of endothelial dysfunction. The lowered NOx levels increased on the 7th day by 66% compared to 4% in the No-HTEMS group and on the 21st day by 75% compared to 65%. A similar positive effect was achieved for the elevated ET-1 level, which decreased in the HTEMS group on the 7th day by 43% compared to 1% in the No-HTEMS group and on the 21st day by 52% compared to 28%. The elevated ADMA level improved with some delay. After 14 days, it declined in the HTEMS group by 53% compared to 27% in No-HTEMS group and on 21st day by 52% compared to 37%.

Potential factors involved in the beneficial action of HTEMS

The beneficial action of HTEMS could be firstly attributed to an improvement of circulatory disturbances. Application of neuromuscular electrical stimulation (NMES) to the thighs enhanced peripheral microcirculation in the intact skin of healthy subjects [39] and improved healing of diabetic ulcers [40]. NMES stimulates the blood flow not only locally, but also at the systemic level. In critical care patients, its application to the lower legs was associated with an enhanced microcirculation of the thenar muscle [13]. There are also observations that acute application of HTEMS to the thighs may increase the GFR [41]. In chronic heart failure patients, continued application of NMES improved endothelial dysfunction and ameliorated microinflammation and muscle strength [42]. Marked circulatory effects are achieved by spinal cord stimulation (SCS). It enhances the blood flow in critical limb ischemia [43] and improves severe angina pectoris (in part via a redistribution of blood flow in the heart) [44].

The NMES-induced circulatory effects are caused in part by an enhanced bioavailability of NO. According to in-vitro and voluntary exercise studies, shear stress is a key activator of eNOS and NO generation [45]. NMES also enhances the insulin sensitivity. This is of particular importance with regard to the insulin resistance in AKI patients [46]. The suppressed NO formation in insulin resistant states is associated with an enhanced ET-1 secretion [47].

In our AKI study, the enhanced ET-1 plasma level decreased significantly after 1 week of HTEMS therapy. This effect could be a consequence of the increased NO formation, which reduces the synthesis of ET-1 [48]. The decline of elevated ET-1 is of special importance in AKI with regard to its strong vasoconstrictive properties [49].

The raised ADMA levels dropped with a delay in the HTEMS-treated patients. Therefore the early positive effects of HTEMS on NO formation occurred independently of ADMA.

Finally, in the beneficial actions of NMES therapy in AKI an improved antioxidant defense could be involved. In rat experiments, even a single session of NMES enhanced antioxidative enzymes and citrate synthase activity [50]. Of special importance seems to be the contraction-induced release of myokines, which mediates local as well as systemic anti-inflammatory actions [8, 51].

Summarizing, our results suggest that intradialytic HTEMS application in patients with AKI enhances the lowered plasma concentration of NOx and decreases the elevated plasma levels of ET-1 and ADMA as compared to AKI patients without HTEMS therapy. With regard to the profound renal vasoconstriction in patients with toxic or intrinsic AKI we hypothesize that the accelerated improvement of parameters of endothelial dysfunction contributed to the shortened clinical course of AKI.

However, our pilot study has important limitations. The number of the included patients is rather low. In particular, with regard of the different causes of AKI, a higher number of patients is necessary. Moreover the severity of AKI is an important determinant of its clinical course. However, in our HTEMS-treated patients, the concentration of C-reactive proteins was significantly higher compared to the No-HTEMS group, suggesting a more severe state of AKI. In future investigations also parameters of protein catabolism should be included, which are important determinants of the clinical course of AKI. In our first study

about the electrotherapy in AKI, we found signs of a decreased protein catabolism in the HTEMS-treated group [20].

Conflict of interests

AH discloses relation with gbo Medizintechnik AG.

References

[1] *Chertow GM, Burdick E, Honour M, Bonventre JV, Bates DW.* Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. J Am Soc Nephrol. 2005; *16:* 3365-3370.

[2] *Ricci Z, Cruz D, Ronco C.* The RIFLE criteria and mortality in acute kidney injury: A systematic review. Kidney Int. 2008; *73:* 538-546.

[3] *Munshi R, Hsu C, Himmelfarb J.* Advances in understanding ischemic acute kidney injury. BMC Med. 2011; *9:* 11

[4] *Goligorsky MS, Brodsky SV, Noiri E.* Nitric oxide in acute renal failure: NOS versus NOS. Kidney Int. 2002; *61:* 855-861.

[5] *Goligorsky MS, Brodsky SV, Noiri E.* NO bioavailability, endothelial dysfunction, and acute renal failure: new insights into pathophysiology. Semin Nephrol. 2004; *24:* 316-323.

[6] *Ratliff BB, Rabadi MM, Vasko R, Yasuda K, Goligorsky MS.* Messengers without borders: mediators of systemic inflammatory response in AKI. J Am Soc Nephrol. 2013; *24:* 529-536.

[7] *Druml W.* Nutritional management of acute kidney injury. In: Kopple JD, Massry SG and Kalantar-Zadeh K (eds). Nutritional Management of Renal Disease, Third edition. London: Elsevier, 2013. p. 605-627.

[8] *Petersen AM, Pedersen BK.* The anti-inflammatory effect of exercise. J Appl Physiol (1985). 2005; *98:* 1154-1162.

[9] *Houmard JA, Tanner CJ, Slentz CA, Duscha BD, McCartney JS, Kraus WE.* Effect of the volume and intensity of exercise training on insulin sensitivity. J Appl Physiol (1985). 2004; *96:* 101-106.

[10] *Calvert JW.* Cardioprotective effects of nitrite during exercise. Cardiovasc Res. 2011; *89:* 499-506.

[11] *Cheema BS, O'Sullivan AJ, Chan M, Patwardhan A, Kelly J, Gillin A, Fiatarone Singh MA.* Progressive resistance training during hemodialysis: rationale and method of a randomized-controlled trial. Hemodial Int. 2006; *10:* 303-310.

[12] *Hamada T, Hayashi T, Kimura T, Nakao K, Moritani T.* Electrical stimulation of human lower extremities enhances energy consumption, carbohydrate oxidation, and whole body glucose uptake. J Appl Physiol (1985). 2004; *96:* 911-916.

[13] *Angelopoulos E, Karatzanos E, Dimopoulos S, Mitsiou G, Stefanou C, Patsaki I, Kotanidou A, Routsi C, Petrikkos G, Nanas S.* Acute microcirculatory effects of medium frequency versus high frequency neuromuscular electrical stimulation in critically ill patients – a pilot study. Ann Intensive Care. 2013; *3:* 39.

[14] *Nuhr MJ, Pette D, Berger R, Quittan M, Crevenna R, Huelsman M, Wiesinger GF, Moser P, Fialka-Moser V, Pacher R.* Beneficial effects of chronic low-frequency stimulation of thigh muscles in patients with advanced chronic heart failure. Eur Heart J. 2004; *25:* 136-143.

[15] *Dobsak P, Homolka P, Svojanovsky J, Reichertova A, Soucek M, Novakova M, Dusek L, Vasku J, Eicher JC, Siegelova J.* Intra-dialytic electrostimulation of leg extensors may improve exercise tolerance and quality of life in hemodialyzed patients. Artif Organs. 2012; *36:* 71-78.

[16] *Gerovasili V, Tripodaki E, Karatzanos E, Pitsolis T, Markaki V, Zervakis D, Routsi C, Roussos C, Nanas S.* Short-term systemic effect of electrical muscle stimulation in critically ill patients. Chest. 2009; *136:* 1249-1256.

[17] *Hamada T, Sasaki H, Hayashi T, Moritani T, Nakao K.* Enhancement of whole body glucose uptake during and after human skeletal muscle low-frequency electrical stimulation. J Appl Physiol (1985). 2003; *94:* 2107-2112.

[18] *Parkington JD, LeBrasseur NK, Siebert AP, Fielding RA.* Contraction-mediated mTOR, p70S6k, and ERK1/2 phosphorylation in aged skeletal muscle. J Appl Physiol (1985). 2004; *97:* 243-248.

[19] *Rose B, Lankisch M, Herder C, Röhrig K, Kempf K, Labrenz S, Hänsler J, Koenig W, Heinemann L, Martin S.* Beneficial effects of external muscle stimulation on glycaemic control in patients with type 2 diabetes. Exp Clin Endocrinol Diabetes. 2008; *116:* 577-581.

[20] *Di Iorio B, Torraca S, Gustaferro P, Fazeli G, Heidland A.* High-frequency external muscle stimulation in acute kidney injury (AKI): potential shortening of its clinical course. Clin Nephrol. 2013; *79 (Suppl 1):* S37-S45.

[21] *Kleinbongard P, Dejam A, Lauer T, Jax T, Kerber S, Gharini P, Balzer J, Zotz RB, Scharf RE, Willers R, Schechter AN, Feelisch M, Kelm M.* Plasma nitrite concentrations reflect the degree of endothelial dysfunction in humans. Free Radic Biol Med. 2006; *40:* 295-302.

[22] *Reichstein L, Labrenz S, Ziegler D, Martin S.* Effective treatment of symptomatic diabetic polyneuropathy by high-frequency external muscle stimulation. Diabetologia. 2005; *48:* 824-828.

[23] *Klassen A, Di Iorio B, Guastaferro P, Bahner U, Heidland A, De Santo N.* High-tone external muscle stimulation in end-stage renal disease: effects on symptomatic diabetic and uremic peripheral neuropathy. J Ren Nutr. 2008; *18:* 46-51.

[24] *Humpert P, Morcos M, Oikonomou D, Schaefer K, Hamann A, Bierhaus A, Schilling T, Nawroth PP.* External electric muscle stimulation improves burning sensations and sleeping disturbances in patients with type 2 diabetes and symptomatic neuropathy. Pain Med. 2009; *10:* 413-419.

[25] *Mian AI, Aranke M, Bryan NS.* Nitric oxide and its metabolites in the critical phase of illness: rapid biomarkers in the making. Open Biochem J. 2013; *7:* 24-32.

[26] *Kwon O, Hong SM, Ramesh G.* Diminished NO generation by injured endothelium and loss of macula densa nNOS may contribute to sustained acute kidney injury after ischemia-reperfusion. Am J Physiol Renal Physiol. 2009; *296:* F25-F33.

[27] *Mian AI, Du Y, Garg HK, Caviness AC, Goldstein SL, Bryan NS.* Urinary nitrate might be an early biomarker for pediatric acute kidney injury in the emergency department. Pediatr Res. 2011; *70:* 203-207.

[28] *Kone BC, Baylis C.* Biosynthesis and homeostatic roles of nitric oxide in the normal kidney. Am J Physiol. 1997; *272:* F561-F578.

[29] *Brodsky SV, Yamamoto T, Tada T, Kim B, Chen J, Kajiya F, Goligorsky MS.* Endothelial dysfunction in ischemic acute renal failure: rescue by transplanted endothelial cells. Am J Physiol Renal Physiol. 2002; *282:* F1140-F1149.

[30] *Vaziri ND, Ni Z, Oveisi F, Liang K, Pandian R.* Enhanced nitric oxide inactivation and protein nitration by reactive oxygen species in renal insufficiency. Hypertension. 2002; *39:* 135-141.

[31] *De Deyn PP, Vanholder R, D'Hooge R.* Nitric oxide in uremia: effects of several potentially toxic guanidino compounds. Kidney Int Suppl. 2003; *63 (s84):* S25-S28.

[32] *Bucala R, Tracey KJ, Cerami A.* Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilatation in experimental diabetes. J Clin Invest. 1991; *87:* 432-438.

[33] *Lundberg JO, Govoni M.* Inorganic nitrate is a possible source for systemic generation of nitric oxide. Free Radic Biol Med. 2004; *37:* 395-400.

[34] *Zoccali C, Bode-Böger S, Mallamaci F, Benedetto F, Tripepi G, Malatino L, Cataliotti A, Bellanuova I, Fermo I, Frölich J, Böger R.* Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: a prospective study. Lancet. 2001; *358:* 2113-2117.

[35] *Kielstein JT, Impraim B, Simmel S, Bode-Böger SM, Tsikas D, Frölich JC, Hoeper MM, Haller H, Fliser D.* Cardiovascular effects of systemic nitric oxide synthase inhibition with asymmetrical dimethylarginine in humans. Circulation. 2004; *109:* 172-177.

[36] *Schramm L, La M, Heidbreder E, Hecker M, Beckman JS, Lopau K, Zimmermann J, Rendl J, Reiners C, Winderl S, Wanner C, Schmidt HH.* L-arginine deficiency and supplementation in experimental acute renal failure and in human kidney transplantation. Kidney Int. 2002; *61:* 1423-1432.

[37] *Ruschitzka F, Shaw S, Gygi D, Noll G, Barton M, Lüscher TF.* Endothelial dysfunction in acute renal failure: role of circulating and tissue endothelin-1. J Am Soc Nephrol. 1999; *10:* 953-962.

[38] *Arfian N, Emoto N, Vignon-Zellweger N, Nakayama K, Yagi K, Hirata K.* ET-1 deletion from endothelial cells protects the kidney during the extension phase of ischemia/reperfusion injury. Biochem Biophys Res Commun. 2012; *425:* 443-449.

[39] *Wikström SO, Svedman P, Svensson H, Tanweer AS; Sven Olof Wikström, Paul Svedman, H.* Effect of transcutaneous nerve stimulation on microcirculation in intact skin and blister wounds in healthy volunteers. Scand J Plast Reconstr Surg Hand Surg. 1999; *33:* 195-201.

[40] *Baker LL, Chambers R, DeMuth SK, Villar F.* Effects of electrical stimulation on wound healing in patients with diabetic ulcers. Diabetes Care. 1997; *20:* 405-412.

[41] *Peckova M, Havlin J, Charvat J, Horackova M, Schück O.* Effects of high-tone external muscle stimulation on renal function in healthy volunteers. Clin Nephrol. 2013; *79 (Suppl 1):* S34-S36.

[42] *Karavidas AI, Raisakis KG, Parissis JT, Tsekoura DK, Adamopoulos S, Korres DA, Farmakis D, Zacharoulis A, Fotiadis I, Matsakas E, Zacharoulis A.* Functional electrical stimulation improves endothelial function and reduces peripheral immune responses in patients with chronic heart failure. Eur J Cardiovasc Prev Rehabil. 2006; *13:* 592-597.

[43] *Brümmer U, Condini V, Cappelli P, Di Liberato L, Scesi M, Bonomini M, Costantini A.* Spinal cord stimulation in hemodialysis patients with critical lower-limb ischemia. Am J Kidney Dis. 2006; *47:* 842-847.

[44] *Mannheimer C, Eliasson T, Augustinsson LE, Blomstrand C, Emanuelsson H, Larsson S, Norrsell H, Hjalmarsson A.* Electrical stimulation versus coronary artery bypass surgery in severe angina pectoris: the ESBY study. Circulation. 1998; *97:* 1157-1163.

[45] *Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM.* Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. Nature. 1999; *399:* 601-605.

[46] *Basi S, Pupim LB, Simmons EM, Sezer MT, Shyr Y, Freedman S, Chertow GM, Mehta RL, Paganini E, Himmelfarb J, Ikizler TA.* Insulin resistance in critically ill patients with acute renal failure. Am J Physiol Renal Physiol. 2005; *289:* F259-F264.

[47] *Kuboki K, Jiang ZY, Takahara N, Ha SW, Igarashi M, Yamauchi T, Feener EP, Herbert TP, Rhodes CJ, King GL.* Regulation of endothelial constitutive nitric oxide synthase gene expression in endothelial cells and in vivo: a specific vascular action of insulin. Circulation. 2000; *101:* 676-681.

[48] *Kelly LK, Wedgwood S, Steinhorn RH, Black SM.* Nitric oxide decreases endothelin-1 secretion through the activation of soluble guanylate cyclase. Am J Physiol Lung Cell Mol Physiol. 2004; *286:* L984-L991.

[49] *Naicker S, Bhoola KD.* Endothelins: vasoactive modulators of renal function in health and disease. Pharmacol Ther. 2001; *90:* 61-88.

[50] *Pimenta AS, Lambertucci RH, Gorjão R, Silveira LR, Curi R.* Effect of a single session of electrical stimulation on activity and expression of citrate synthase and antioxidant enzymes in rat soleus muscle. Eur J Appl Physiol. 2007; *102:* 119-126.

[51] *Brandt C, Pedersen BK.* The role of exercise-induced myokines in muscle homeostasis and the defense against chronic diseases. J Biomed Biotechnol. 2010; 520258.