Effect Of Monochromatic Infrared Energy on Quality Of Life And Intra-Epidermal Nerve Fibre Density In Painful Diabetic Neuropathy: A Randomized, Sham Control Study

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Abstract

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Background: Monochromatic infrared energy (MIRE) is a non-invasive modality that has evoked mixed results for symptomatic relief of painful diabetic neuropathy. However, intra-epidermal nerve-fiber density (IENFD) considered as the gold standard for small-fiber neuropathy has not been studied following MIRE therapy.

Research design and methods: Participants with type 2 diabetes and painful DPN were randomized to receive MIRE or sham therapy dosed thrice a week for 12 weeks. Quantitiave assessment of IENFD was performed from 3mm skin punch-biopsy specimens at baseline and after 12 weeks. We also assessed the quality of life (QOL) with Norfolk QOL, symptom severity with visual analogue scale (VAS), and objective neuropathy assessment with Michigan Neuropathy Severity Instrument (MNSI), neuropathy disability score (NDS).

Results: Thirty-eight participants were enrolled and 23 completed the study protocol (15 in each group). The mean age of participants in MIRE cohort was 59.1±9.2 years, duration of diabetes 12.9±3.1 years and painful symptom were for 3.9±3.7 months. The mean IENFD was 0.90±0.73/mm^2 ($p<0.01$) and 1.71±1.11/mm^2 in the MIRE cohort and 0.60±0.89/mm^2 and 2.17±0.98/mm^2 ($p<0.01$) in sham cohort at baseline and after 3 months. The median decline in VAS was 5.1 (4.0-7.6) and 3.0 (0.4-5.6) points (intergroup difference, $p=0.01$); and an increase in Norfolk QOL-DN by 15 (11-18) and 4 (4-14.2) points (intergroup difference, $p=0.021$) in MIRE and sham cohort, respectively after 3 months.

Conclusions: MIRE therapy provides symptomatic benefit and improve quality of life in patients with painful diabetic neuropathy. However, MIRE therapy do not increase IENFD over short term.

Highlights:

1. Treatment of neuropathic pain in people with diabetes is difficult despite numerous pharmacological agents.
2. This article assessed the effect of monochromatic infrared light (MIRE) on pain symptoms, quality of life and intra-epidermal nerve-fiber density (IENFD) in chronic diabetic neuropathic pain.
3. MIRE therapy is effective for short term relief of painful symptoms and improving quality of life.
4. MIRE therapy does not increase IENFD to have any significant beneficial effect on nerve regeneration.

Key words: Diabetic Neuropathy; Painful Diabetic Neuropathy; Monochromatic Infrared Energy; Quality of Life; Norfolk QoL

Introduction:

About half of the people with diabetes are affected with peripheral neuropathy during their lifetime.[1] Distal symmetrical polyneuropathy (DSPN) is by far the most common type of diabetic neuropathy, which affects more than 90% of the patients.[1,2] DSPN is usually confirmed by evaluating large-fibre sensations with vibration perception or nerve conduction studies. However, presence of small-fibre neuropathy portends an equal or more significance, as structural and functional small fibre changes precinct large fibre changes and are causative for neuropathic pain, loss of protective sensation and foot ulceration.[2-5]

Neuropathic pain may be the first presentation of diabetes that brings the patient to medical facility. Neuropathic pain in people with diabetes is distinguished by burning, tingling, lancinating, sharp, and shooting or even as shock like sensation, generally worse at night and may cause insomnia.[1, 2, 5] The pain can be persistent and associated with cutaneous allodynia, that influences the ability to perform activities of daily living which can markedly affect the quality of life. As a result, people affected with neuropathic pain have depressed mood, refrain from social and recreational activities that exacerbates their disability.[6,7]

A wide variety of drugs, used alone or in combination, have been shown to reduce neuropathic pain compared with placebo in randomized controlled trials including anticonvulsants, antidepressants, opioids, capsaicin topical cream, lidocaine patch, alpha lipoic acid, isosorbide dinitrate spray etc.[8-11] The management of painful peripheral neuropathy consists of excluding other treatable causes, improving glycemic control and medications to alleviate pain, but pain relief remains inadequate for most patients.[11,12] The pharmacological treatment for diabetic neuropathy is predominantly targeted at symptomatic relief and not focused on the pathophysiological mechanism, limited by side effects, marked by the development of tolerance and significantly add to economic burden of diabetes treatment.[9, 11, 13]

Adjunctive therapies including varioustypes of electrotherapy like transcutaneous electric nerve stimulation (TENS), static magnetic field therapy, pulsed electromagnetic field (PEMF), external muscle stimulation have been used in neuropathic pain of varied etiologies and shown to decrease pain.[14,15] Monochromatic infra-red energy (MIRE) is another such therapy that has been used for DPN earlier and approved by United States Food and Drug

Administration (FDA) in 1994 for alleviating pain and increasing circulation.[16] Few initial studies that evaluated MIRE therapy demonstrated a significant decrease in neuropathic pain (17-20) and improvement in tactile sensitivity[17-21], while others have not shown any change in symptom scores [22,23] or plantar sensitivity [22-25] post MIRE therapy. However, studies with MIRE therapy had methodological limitations as they were retrospective, lacked a control group, treatment was not supervised and were of short duration (2-4 weeks).[26] Also, most of the prior studies evaluated subjective outcome measures of neuropathy, thus subject to inherent bias. Intra-epidermal nerve fiber density (IENFD) which is an objective and earliest marker of small fiber neuropathy that is implicated for neuropathic pain and LOPS in people with diabetes has not been studied earlier. Therefore, we assessed the effect of MIRE therapy on IENFD and also symptom relief and quality of life and in patients with DPN.

Participants and Methods:

Patients with self-reported diabetes presenting to outpatient department of tertiary referral centre constituted the reference population. The study was incited after the approval of the Institutional Ethics Committee and was conducted along the lines of the declaration of Helsinki. Participants of either gender, age 18 years and above, on stable oral hypoglycemic drugs and/or insulin for the preceding three month with history and clinical features of painful DPN, and meeting the inclusion and exclusion criteria were included in the study after an explained and written informed consent. The diagnosis of painful diabetic neuropathy was based on symptoms & signs of small fibre neuropathy as defined by the "Toronto Diabetic Neuropathy Expert Group" recommendations that include diabetic neuropathy symptom score (DNS) of >2 out of a maximum of 4, or b) Neuropathic disability score (NDS) of >6 out of 10.[27] Participants with peripheral vascular disease (Ankle-Brachial Indiex <0.9 and/or claudication), chronic kidney disease (eGFR<30 ml/min/m^2), depression, malignancy, knee and back surgeries dermatological diseases like psoriasis, leprosy, eczema and connective disuse disorders, retropositive, familial neuropathies, on medications associated with peripheral neuropathy including anti-tubercular therapy, anti-retroviral therapy,and pregnant or lactating women were excluded from the protocol.

All patients were engaged in an initial 2-week run-in period in order to ensure their compliance for follow up visits after the withdrawal of pre-existing medication for painful DPN. Paracetamol was used as a rescue medication, if needed. After the run-in period of two weeks, patients were allocated MIRE (active) or sham (placebo) therapy in 1:1 simple randomization design. The groups were coded A (MIRE) and B (sham) in two envelopes and the participants were asked to pull out the envelope for group allocation. The investigator, the

patient and the outcome assessor were blinded to treatment allocation and envelope was disclosed to the technician responsible for delivering the therapy, who was not involved in design of the study or data evaluation.

Clinical examination:

The feet were examined for presence of dryness of skin,presence of callus, ulcer formation,fissures or deformities including pes planus, toe hammer, overlapping toes, hallux valgus, subluxation of joints, prominence of metatarsal heads, bunion, medial convexities or prior amputation.

Study and sham cohorts were screened with Diabetic neuropathy score (DNS) and Neuropathy disability score (NDS) and further evaluated with Michigan neuropathy screening instrument (MNSI) which consists of symptom questionnaire (MNSI-Q) and clinical examination (MNSI-P) scores.[28] The MNSI questionnaire provides a graded response of neuropathic symptoms, so that, a higher score represents more neuropathic symptoms with a maximum of 25 (15-history,10-physical examination).

A 10 grams (5.07) Semmes Weinstein monofilament test was employed for testing loss of protective sensation as a part of MNSI clinical examination and vibration perception threshold (VPT) using biothesiometry [Vibrometer-VPT® (Diabetic Foot Care, Madras Engineering Service, India)]. A 10-g Semmes-Weinstein monofilament, SWM (A+Elite 3-in-Retractable Pocket Pen Semmes Weinstein Monofilament, USA) was placed at right angles to the skin and pressure was applied until the filament just bent with a mean contact time of 1 second as a part of MNSI-physical examination. Monofilament was applied to 10 sites mainly great toe,3rd, 5th toes,1st,3rd and 5th heads of metatarsals, medial and lateral parts of mid-foot, heel region and over dorsal aspect between 1st and 2nd toes. Vibration sensation was tested with a 128 Hz tuning fork placed at the distal plantar surface of big toe of both legs. The response was considered abnormal when the patient reported loss of vibratory sensation while the examiner still perceived it.

After familiarization, vibration perception threshold (VPT) was also assessed at the distal part of the plantar surface of great toe of both the legs, first, third and fifth metatarsal bases, heel and midsole. The voltage was gradually increased at the rate of 1 mV/sec and the VPT score was denoted as the level of voltage when the patient indicates that they first perceived the sense of vibration. The mean of six records was obtained and neuropathy was considered if VPT was $\geq$ 25mV (at any site). VPT of both limbs was recorded before and after therapy. Ankle reflex were examined using an appropriate reflex hammer.

Quality of life parameters

Norfolk QOL-DN instrument is a validated nerve fibre-specific questionnairefor evaluating the overall QOL by categorising symptoms and their eventual impact on the functional status and activities of daily living of patients with peripheral neuropathy.[29] The questions also address small and large somatic nerve fibres and autonomic nerve function. The total Norfolk QOL-DN score embraces symptoms of neuropathyand the neurologic examination of motorfunction, sensory perception, and reflexes. A 10-point visual analogue scale (VAS) was used to assess the discomfort due to painful diabetic neuropathy.

Skin biopsy for IENFD

A 3mm skin punch biopsy was performed 10 cm proximal to lateral malleolus in the limb which scored worst on former evaluation tools. The skin biopsy specimen was divided into two halves for Haematoxylin & Eosin(H&E) stain and immunohistochemistry (IHC)/immunofluorescence (IF). Tissues sections were fixed in 10% buffered formalin processed and embedded in paraffin, following a standard protocol for H&E stain.The other half was stored at -20 degree Celsius for IHC/IF. Sections were cut at 50 µm sections on pre coated slides with poly-L-lysine. The paraffin/frozen sections were de-paraffinised in xylene using three changes for 5 minutes each. Sections were then hydrated gradually through graded alcohols by washing in 100% ethanol twice for 10 minutes each, then in 95% ethanol twice for 10 minutes each and lastly in de-ionised H_2O for one minute with stirring. De-paraffinised sections were exposed to microwave fixation at 750W for 10min to increase immunogenicity. The sections were again washed in de-ionised water three times for 2 minutes each.

The slides were incubated for 5-10 minutes in 1% hydrogen peroxide in de-ionised H_2O to quench endogenous peroxides activity and then were washed in phosphate buffered saline (PBS), pH 7.2 twice for 5 minutes each. Incubation was done for 1 hour in 1.5% normal blocking serum in PBS to inhibit non-specific binding. The slides were incubated with primary antibody –protein gene product 9.5 (PGP 9.5) (Abcam,UK) a neuronal ubiquitin carboxy terminal hydrolase, for 30 minutes at room temperature or overnight at 4^{o} C. Optimal antibody concentration was determined by titration per the manufacturer's instructions. The slides were then washed with three changes of PBS for 5 minutes each.

Incubation for 30 minutes was done in peroxidise substrate till the desired stain intensity developed (5 minutes). Individual slides were then monitored to determine the proper development time. Then sections were washed in de-ionised H_2O for 5 minutes. Counter-stain with haematoxylin for 20 seconds followed by immediate washing with several changes of de-ionised H_2O. Dehydration through alcohols and xylene were done as follows:

Soaking in 95% ethanol twice for 10 seconds each, 100% ethanol twice for 10 seconds each, then xylene three times for 10 seconds each. Excess xylene was wiped off. Immediately 1-2 drops of permanent mounting medium i.e. di-n-butyl phthalate in xylene (DPX) was added and cover slides with glass cover slip and observe under light microscopy.

Quantification of IENFD was done by counting the density of nerve-fibres that cross the dermo-epidermal junction per 1 millimetre in at least 2-3 non-adjacent sections procured from different sites within the specimen.

Treatment procedures:

A single MIRE unit was used in this study -Anodyne Model 480– Infrared Therapy System (Medassist, Tampa, FL). The device has a main power unitwith four flexible therapy pads; each padmeasures 3.0 x7.5 cm and contains 60super luminousgallium-aluminiumarsenidediodes that emit light energy in the near-infrared spectrum (890-nm wavelength). The active treatment unit was set to deliver 1.95 Joule/ cm^2/ min when activated.

For sham therapy, the pads of the MIRE device were applied to the foot similarly, but the switch was not activated and the patient was blinded to thisinformation. The illuminating bars on the display were covered with black cellophane tape beforehand. Each subject sat in a standard chair after removal ofsocks and shoes. The four therapy pads were placed over the following sites *1*) Distal posterior leg, *2*) Distal anterior leg *3*) Plantar footover metatarsal heads, and *4*) Plantar arch of foot.

The placement of pads 3 and 4 formed a “T” on the plantar surface of the foot. Commercial plastic wrap was placed between the skin and the MIRE pads for hygienic purposes, and the pads were held in position with neoprene straps supplied by the manufacturer. Therapy was given for 30 min a session, 3 sessions per week for a total of 36 sessions over 12 weeks. So a total of 36 sessions were given to an individual (both cases and controls). Patient was deemed compliant if he/she receives 80% or above of 36 sessions.

Patients in both cohorts were subsequently evaluated for change inVAS score, Norfolk QOL-DN questionnaire scores,VPT, MNSI scores, and IENFD after intervention. Patient who attended 80% of the total allotted sessions of MIRE to be included in the analysis.

Statistical analysis:

Mean ±SD was used for parametric data and median with inter-quartile range was employed as measure of central tendency for non- parametric data. Baseline parameters were compared by unpaired or paired student T test, Chi-square test or Fisher exact test. The patient’s VAS, Norfolk QOL-DN questionnaire scores, MNSI-questionnaire and physical exam scores, VPT

score, NDS and DNS scores were compared between the groups using the Mann- Whitney and Wilcoxon matched pair test to evaluate the changes in cohort scores before and after therapy. In addition, data from all visits was compared with baseline levels. A p-value <0.05 was taken as significant. SPSS version 22 was employed to perform statistical analysis.

Results: Eighty patients were screened, of which 45 patients satisfied the inclusion criteria. Seven patients did not provide the consent; hence 38 patients went through the run-in period. Thirty patients were randomized as four patients did not report after run-in period, two withdrew consent and two patients could not tolerate the drug withdrawal phase in view of intolerable neuropathic symptoms. The baseline characteristics of the 30 enrolled participants are shown in Table 1.The mean duration of diabetes was 12.9±3.1 years and duration of painful DPN symptoms was 3.9±3.7 years in the MIRE cohort.

The median baseline VAS was 9.0 (7-9.6) and 6.9 (6.5-8.8), p=0.106 in MIRE and sham cohorts, respectively. VAS scores at the end of 1st, 2nd and 3rd month of MIRE therapy were 5.3(4.2-7.7),4.5(3.2-6.1) and 2.5 (0.9-4.8) points, respectively (Figure 1) with an overall 56.7% decrease in VAS score. There was a decrease in VAS score by 3.1(2.6-5.8) points and 2.4 (0.6-2.7) points, (intergroup difference, p=0.01) at 2 months and 5.1 (4-7.6) points and 3 (0.4-5.6) points, (intergroup difference, p=0.04) at the end of three months in the MIRE and sham cohort , respectively. The median Norfolk QOL-DN score were 24 (17-27) and 23 (13.5-25), p=0.48 at baseline and 8 (6-12) and 12 (8.2-21), p=0.07 at the end of 3 months in MIRE and sham cohorts, respectively (Figure 2). The Norfolk QOL-DN score increased by 15 (11-18) points after MIRE therapy and 4 (4-14.2) in sham cohort (intergroup difference, p=0.021).

The median baseline DNS and NDS scores were 3.0 (2.0-3.0) and 8.0 (7.0-8.0) in the MIRE cohort. No change in DNS (intergroup difference p=0.47) and NDS scores (intergroup difference p=0.51) were observed after 3 months of MIRE or sham therapy. The median MNSI-Q and MNSI-P scores at baseline were 6.0 (5.0-8.0), 4.0 (3.0-4.0) and 5.0 (5.0-6.0) and 3.5 (2.2- 5.0) in the MIRE cohort and sham cohort, respectively. Post therapy at 3 months, a decline in MNSI-Q of 3 (2-4) and 2 (-0.5-2.7)points (intergroup difference, p=0.06) was observed in MIRE cohort and sham cohort, respectively. However, no change in MNSI-PE scores was observed with either treatment at the end of 3 month. The IENFD was 0.90±0.73 mm, 0.6±0.89 mm (p=0.43) at baseline; and 1.71± 1.11 and 2.17±0.98 mm, (p=0.45) after three months of therapy in the MIRE and sham cohort, respectively. There was an increase in IENFD of 1±1.26 and 2 ±1.15mm (p=0.22) with MIRE and sham cohort, respectively at the end of three months (Figure 3).

Discussion:

The results of the present sham-control study suggests a significant decrease in symptoms of neuropathic pain as assessed by visual analogue scale and improvement in quality of life scales as assessed with Norfolk QOL scores with the use of MIRE therapy. However, there was no significant change in the objective measures of neuropathy including VPT, DNS, NDS scores, MNSI questionnaire and physical examination scores. Also, an increase in IENFD was observed with MIRE therapy, but IENFD change was comparative to sham treatment at the end of twelve weeks.

Generally, in clinical trials with pharmacological agents for DPN, the treatment is considered successful if patient would obtain 50% of reduction in the pain level associated with some additional beneficial effects on sleep, fatigue, depression and quality of life. Previous studies have demonstrated a 37.1%-45.2% decrease in pain on 10-point or 100-point VAS scale with 4-12 weeks of MIRE therapy.[17-20] We observed a 56.7% decrease in pain scores on 10-point VAS scale with MIRE therapy, along with a significant improvement in QOL. Higher symptomatic benefit in our study could be because of longer duration of MIRE therapy (12 weeks) and a higher baseline VAS score (median VAS of 9.0) as compared to the previous studies[17-20] which have used MIRE therapy for 4-6 weeks and lower baseline VAS. Contrary to these results, prior study by Lavery et al did not observe any effect of MIRE therapy on neuropathic pain but had certain methodological limitations as it was based on domiciliary MIRE therapy and not a supervised intervention.[22] A recent meta-analysis for randomized control studies with MIRE therapy showed no difference between MIRE and comparison groups [mean difference 0.80 (95% CI: 0.30-0.68)]. [26] However, the meta-analysis did not include recent study by Ammar TA[21] that showed significant decrease in neuropathic pain.

MIRE therapy has also been previously evaluated for its effects on the restoration of protective sensations with the assessment of SWS monofilament perception before and after therapy. The results were heterogenous as few authors have shown a significant (66-80.5%) improvement in foot sensation as assessed by the number of sites sensate to SWM monofilament [17-21], while others have not. [22,24,25] We also observed no apparent change in SWM sensation, VPT score and overall NDS and DNS scores with MIRE therapy. A recent meta-analysis has demonstrated no increase in plantar regions sensitive to 5.07 SWM compared to the sham cohort [standardized mean difference =0.22 [95% CI,: -0.07to 0.51)]. But, a subgroup analysis showed improved sensations with 2 weeks of MIRE therapy but the effect were not sustained for the next 2 weeks. [26] Possibly, the duration of therapy is too short

to alter the vasculature, blood flow and nerve regeneration. Also, predominantly large fibres are tested with VPT which are unlikely to be affected by photoemissions.

The quantitative estimation of small nerve fiber damage by evaluating IENF density is pivotal for an early diagnosis of diabetic neuropathy and is considered a sensitive modality for assessing progression or regression after any intervention. [30,31] IENFD also has a level A recommendation by the European Federation of the Neurological Societies and the Peripheral Nerve Society as a reliable and independent method to confirm the clinical diagnosis of small fibre neuropathy. [32] Interventions like supervised exercise has been shown to improve cutaneous nerve fibre branching in people with diabetic peripheral neuropathy; however, IENFD has not been previously studied with MIRE therapy. [33,34] MIRE therapy is expected to improve the cutaneous nerve fiber density by an increase in blood flow through the generation of nitric oxide in vasa nervosum. We studied the density of unmyelinated nerve fibres crossing the dermo-epidermal junction as a robust marker of small fiber diabetic neuropathy. IENFD at baseline in our cohort was similar to that reported by Smith et al and Boucek et al suggesting adequate sampling and procedure for nerve fiber density assessment. [35,36] We observed a non-significant increase in IENFD in both MIRE at the end of three months with no intergroup differences. The plausible explanation for the lack of efficacy of intervention could be a longer duration of diabetes in our cohort and it is observed that the duration of diabetes is an important determinant of IENFD loss. A 1mm loss of nerve fiber per year is observed in patients with diabetic neuropathy contributing to severe loss may with increasing duration of diabetes that may be irreversible with short-term interventions. Also, short duration of MIRE therapy precludes sensitivity of IENFD estimation.

Peculiarly, there was a strong placebo effect in our study and most of the other previous randomized controlled studies. [17-19] “Hawthorne effect” may explain the findings in uncontrolled studies which showed unequivocal effectiveness of MIRE therapy. [37] Also, precision and accuracy in assessing neuropathy is hindered by lack of reliable, objective and non-invasive tools for longitudinal testing and the placebo effect on subjective parameters might take leverage of this defect. An improvement in QOL in sham cohort could be due to reinforcement in regular follow-up visits and regular counselling by health care professional. However, the improvement in IENFD with sham therapy might be due to technical difficulties including folding of skin samples and excessive background noise obscuring the nerve fibres and lack of dedicated software for counting IENFD, which may have affected IENFD count in both sham and MIRE cohorts.

The strengths of the study include being the first randomized, sham controlled study to evaluate IENFD in patients with painful diabetic neuropathy in a close four weekly follow-up protocol with a good adherence to intervention. Further, multiple established rating scales were used to assess symptomatic changes in painful diabetic neuropathy. Some of the limitations perceived include a small sample size due to number of patients not tolerating the withdrawal of pharmacological therapy for painful neuropathy contributing to inadequate power of the study, and technical difficulties inprocessing skin biopsy for IENFD leading to wastage of considerable fraction of nerve biopsy samples. The duration of therapy in the present study was 12 weeks that might not be appropriate to have any perceptible changes in quantitative neuropathy assessment index of IENFD.

In conclusion, MIRE is associated with a significant symptomatic improvement and better quality-of-life in patients with painful diabetic peripheral neuropathy. However, MIRE therapy do not result in significant increase in cutaneous re-innervation over twelve weeks of therapy.

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Contributions: PU was involved in clinical care of the participants, did biopsy for IENFD and wrote the initial draft of the manuscript; AR was involved in the design of the study, recruitment, clinical care and follow up of the participants, writing and editing the manuscript; AB was involved in the design of the study, and editing the manuscript; UNS performed the histopathology of tissue specimen and edited the manuscript.

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Figure Legends:

Figure 1: Comparison of visual analogue scores between MIRE and sham cohort

Figure 2: Comparison of NOEFOLK Quality of Life scores between MIRE and sham cohort

Figure 3: Comparison of Intra-epidemal Nerve Fiber Density (IENFD) between MIRE and sham cohort

Table 1: Comparison of baseline characteristics between two cohorts

Parameters	**MIRE COHORT N=15**	**SHAM COHORT N=15**	**P-value**
Age (years)	**59.1±9.2**	**59.3±10.1**	**0.965**
Female Gender (%)	**53.3**	**75.0**	**0.006***
BMI kg/m^2	**27.1±5.6**	**27.6±4.4**	**0.830**
HbA1C (%)	**7.2±0.8**	**7.1±0.7**	**0.637**
Duration of diabetes (years)	**12.9±3.1**	**11.4±4.4**	**0.352**
Duration of neuropathic pain symptom (months)	**3.93±3.74**	**1.43±0.82**	**0.055**

Data is shown as mean±SD, unless specified

*P<0.05 was considered significant

BMI: Body Mass Index