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1 **Resistance Training improves Nerve Conduction and Arterial Stiffness in Older Adults**

2 **with Diabetic Distal Symmetrical Polyneuropathy: A Randomized Controlled Trial**

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22 **Word Count:** 4516

23 **Number of Tables:** 2

24 **Number of Figures:** 2

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28
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Abstract

Diabetes is the main cause of peripheral neuropathy where older patients are at increased risk of diabetic distal symmetrical polyneuropathy (DSPN) due to age-related nerve degeneration and vascular changes. The aim of the study was to investigate the effect of resistance training on nerve conduction, measures of neuropathy and arterial stiffness in older patients with DSPN. In a randomized controlled trial, thirty-four older adults with type-2 diabetes and peripheral neuropathy were enrolled and randomly assigned to experimental and control groups. The experimental group carried out circuit resistance training (1-3 rounds, 11 exercises, 10-15 reps, 50%-60% of 1RM, 3 times per week) for 12 weeks. Measurements were performed at baseline and 48 h after the intervention. Measures of DSPN including Michigan neuropathy screening instrument (MNSI), Michigan diabetic neuropathy score (MDNS), motor nerve action potential amplitude (APA), sensory and motor nerve conduction velocity (NCV) improved following intervention ($p < 0.001$, $p = 0.001$, $p = 0.034$, $p = 0.001$, and $p = 0.001$, respectively). Sensory APA did not change after the intervention ($p = 0.139$). Cardio-ankle vascular index (CAVI) and ankle-brachial index (ABI) improved in the experimental group compared with the control group ($p = 0.014$ and $p = 0.033$, respectively). In addition, HbA1C decreased following the 12-week resistance training program ($p = 0.002$). Older adults with DSPN respond positively to resistance training by improved neuropathy symptoms, nerve conduction, arterial stiffness and glucose regulation. Resistance training offers a positive intervention that can abate the progression of DSPN in older adults.

Key words: Type-2 diabetes mellitus; Nerve conduction; Polyneuropathy; Arterial stiffness; Exercise training.

1. Introduction

Type 2 diabetes mellitus is associated with progressive neurodegenerative complications. Diabetic distal symmetrical polyneuropathy (DSPN) is a chronic microvascular complication of diabetes that can cause symptoms of severe pain and numbness [1]. Metabolic and vascular factors are known to be the major contributors of the pathogenesis and development of DSPN [2]. Accordingly, elevated glucose concentration and impaired vascular function is associated with severe neuropathy [2]. For instance, elevated femoral arterial stiffness has been reported to be associated with ischemic symptoms in the lower extremity of patients with diabetes [3]. Increased arterial stiffness in the lower limbs is accompanied by elevated resistance against blood flow that leads to decreased flow in the limbs arteries [3]. Diabetes related peripheral neuropathy is especially troublesome in older patients as aging is an independent risk factor for peripheral neuropathies [4]. Aging is associated with structural and functional changes in the central and peripheral nervous system [5]. It has been indicated that aging can increase nerve lipid peroxidation, Schwann cell apoptosis [6], nerve demyelination and axonal degeneration [7]. Hence, older patients with DSPN are at increased risk of nerve degeneration and related complications [4]. These changes have a negative impact on activities of daily living and quality of life and increase the risk of morbidity and mortality in this population [4, 8]. DSPN is the major cause of foot ulceration and non-traumatic lower limb amputation in diabetes [9]. Thus, any measures that can lessen the signs and symptoms of the disease would be a welcome adjuvant intervention.

Currently, there is no definitive treatment for DSPN and the majority of therapies focus on pain abatement [10]. However, addressing the underlying mechanisms of DSPN including hyperglycemia and vascular dysfunction can influence the development of the disease [10]. Optimizing glucose control has been shown to be crucial for preventing and delaying the development of peripheral nerve damage in type 2 diabetes [11]. In addition, restoring blood

86 flow to the lower extremity improves nerve conduction in patients with DSPN [12]. Exercise

87 training offers a wide range of benefits to patients with type 2 diabetes including optimal

88 glycemic control, improved vascular function and arterial stiffness [13-15]. Additionally,

89 physical activity and exercise training are known to have benefits on central and peripheral

90 nerves function. For example, aerobic training protects peripheral nerves from pathological

91 changes related to aging [6] and strength training has been shown to increase the thickness of

92 myelin sheath and delay age-related progressive changes in peripheral nerves [16].

93 Evidence shows that exercise has benefits on growth of damaged nerves that is suggested

94 to be dependent on the type of injury, exercise modality, duration and intensity [17, 18].

95 However, there is little consensus on the benefits of different exercise modalities on diabetes-

96 related peripheral nerve damage due to the paucity of research. Aerobic and balance exercises

97 are frequently studied and suggested modes of exercise for patients with DSPN [19]. Reviews

98 of literature indicated that resistance training has been recommended for clinical purposes as it

99 brings about various benefits [14, 15]. For instance, some studies suggested that resistance

100 training can be more effective than aerobic training for glucose regulation in patients with type

101 2 diabetes [13]. These benefits can have unique clinical implications in type 2 diabetes and the

102 related complications. However, to date, no study has evaluated the effect of resistance training

103 on DSPN in older adults, which might be related to the concerns regarding feasibility and safety

104 of exercise in this population. Physicians seem to be reluctant to encourage patients with DSPN

105 to engage in resistance-type exercise, possibly because of fears of adverse outcomes such as

106 increased pain or foot ulceration. This may have been arisen from preliminary research that

107 suggested DSPN might be a contraindication for weight-bearing exercise [20]. However, recent

108 studies indicated that weight-bearing exercise in the form of aerobic and balance exercise do

109 not increase foot pain and ulcers in individuals with DSPN [21]. Accordingly, the aim of this

110 investigation was to investigate the effect of supervised low-to-moderate intensity resistance-

111 type exercise program on neuropathy symptoms, nerve conduction and arterial stiffness in older

112 adults with DSPN. We hypothesized that engaging in resistance training would have a positive

113 influence on neuropathic symptoms in older adults with DSPN.

114

115 **2. Materials and Methods**

116 A randomized-controlled trial was conducted to assess the effect of a 12-week resistance

117 training on neuropathy symptoms, nerve conduction and arterial stiffness. The study complied

118 with the codes of *Declaration of Helsinki* for human research and approved by the Human

119 Research Ethics Committee of Shahrood University of Medical Sciences

120 (IR.SHMU.REC.1398.087, 09.30.2019) and the trial was registered at Iranian Clinical Trial

121 Registry (Trial No: IRCT20170120032066N4); although the trial appears to be registered

122 retrospectively, all authors can attest that the trial was registered prior to participant enrolment.

123

124 *2.1. Participants*

125 Community-dwelling male patients registered in diabetes clinics and aged > 60 years were

126 invited to participate and screened for eligibility. The patients were included if they had history

127 of diabetes > 5 years, HbA1c > 6.6%, inactive lifestyle, and if they were diagnosed with mild

128 to moderate stage of DSPN. Participants were excluded if they were not permitted to participate

129 in exercise programs, if they had orthopedic issues, foot deformity and ulcers, and if they had

130 absent nerve action potential amplitude. The diagnosis of type 2 diabetes was based on the

131 criteria by American Diabetes Association and the presence of DSPN was confirmed by a

132 neurologist based on Michigan Diabetic Neuropathy Score (MDNS) and Michigan Neuropathy

133 Screening Instrument (MNSI) with signs and symptoms of DSPN [22, 23]. Physical activity

134 level was assessed by The International Physical Activity Questionnaire (IPAQ) and all the

participants were leading inactive lifestyle. Eligibility was confirmed in 34 volunteers (Figure 1), they were informed about the study procedure and provided written consent.

2.2. Randomization

The randomization was carried out by a person independent of the trial team to ensure concealment of the allocation. The participants were assigned to the groups using a blocked/ stratified randomization method in a 1:1 ratio. Stratification was applied based on the stage of DSPN at two levels including mild and medium neuropathy. A distinct block randomization with the block size of 4-6 was implemented for each stratum. The participants were randomly assigned to each group using random number tables generated by computer.

2.3. Exercise Training

According to exercise guidelines, type 2 diabetic patients have been recommended to engage in resistance training programs with moderate intensity of $\geq 50\%$ of 1RM at least 2 times a week on non-consecutive days [24]. Given this and previous pilot studies in this population, the resistance training protocol began at the least recommended exercise intensity and preceded by a familiarization period. The experimental group was familiarized with the exercise protocol over two weeks. One-repetition maximum (1RM) was estimated by each participant performing 10-RM for each exercise. This was re-assessed every month to adjust the resistance for each exercise in order to ensure progressive overload. The resistance exercise program was performed 3 times per week, for 12 weeks that lasted ~90 minutes per session and consisted of 11 dynamic exercises on large muscle groups with free weights and machines including squat, bench press, shoulder press, knee extension, knee flexion, calf rise, elbow extension and flexion, sit ups, lat pulldown and back extension. The resistance training consisted of 1-3 circuits with 10-15 reps for each exercise at between 50-60% of 1RM. Rest intervals of 30-60

160 s between each exercise and 3-5 min between each round were allowed. A 15-minute warm up

161 and 10-minute cool down consisting of brisk walking/ jogging, stretching of major muscle

162 groups and the main exercises with lighter weights were included at each session.

163   All exercise sessions were carried out under the supervision of an exercise physiologist.

164 To avoid any possible hyper/hypoglycemia induced by exercise, blood glucose levels were

165 checked prior to each exercise session to ensure participants were in a safe range (not to be <

166 100 mg/dl or > 250 mg/dl). If glucose testing revealed hyperglycemia, exercise training was

167 postponed and if hypoglycemic, a carbohydrate snack was provided and glucose was re-

168 checked. Furthermore, to avoid DSPN-related adverse events such as pain aggravation and foot

169 ulceration, the participants were asked to be wearing comfortable and dry socks and shoes

170 while exercising.

171   Participants were required to follow their habitual diet throughout the study. During the

172 familiarization period and during the last week of training, the calorie intake was recorded by

173 a 3-day dietary recall. Dietary data were recorded in the case of significant difference between

174 groups and/ or between pre and post-test and hence requiring the application of a covariate of

175 analysis. In addition, food intake before baseline measurements was recorded using a 24-hour

176 recall and participants were asked to replicate the same diet before the final measurements to

177 the best of their ability.

178

179 *2.4. Measurements*

180 In line with our hypothesis the primary outcomes were MDNS, Sural sensory and Peroneal

181 motor NCV and action potential amplitude, CAVI and ABI. All measurements were carried

182 out at baseline and were replicated 48 h following last exercise session. The participants were

183 asked to abstain from vigorous physical activity and caffeine for 24 h before the measurements.

184 Body composition was analyzed by a bioelectrical impedance analyzer (Inbody230, Korea)

185 after an overnight fast. Fasting blood samples were taken from the antecubital vein and used to

186 assess HbA1c content by liquid chromatography (Pishtaz, Tehran, Iran). Arterial stiffness was

187 measured by oscillometric method using VaSera VS-2000 (Fukuda Denshi, Tokyo, Japan) that

188 captures pulse waves in 4 limbs non-invasively. Before measurements, participants

189 comfortably rested supine for 10 minutes. The measurements were carried out according to the

190 manufacturer's instruction. Blood pressure cuffs with sensors were applied to all limbs to

191 generate plethysmograms. Phonocardiogram (PCG) was also placed at sternal angel and ECG

192 leads were attached to the arm. The measurements of brachial and ankles systolic pressure and

193 calculations of CAVI and ABI were automatically carried out by the Vasera-2000 system [25].

194 Nerve conduction studies and neuropathy assessments were performed by a neurologist blinded

195 to group allocations using an EMG/NCV system (Viking Quest, USA). For sural sensory nerve

196 the recording point was behind the lateral malleolus and stimulation point was 14 cm from the

197 active electrode up to the posterior aspect of the calf. For peroneal motor nerve recording point

198 was at extensor digitorum brevis muscle and stimulation points were at anterior ankle and

199 lateral popliteal fossa immediately below the head of fibula. For both nerves studied nerve

200 action potential amplitude (APA) and nerve conduction velocity (NCV) have been reported.

201 The measurements were performed in a quiet room with constant temperature 25 ± 2 °C.

202 Michigan Diabetic Neuropathy Instrument (MNSI) consists of 15 questions with yes/no

203 responses on foot sensation that indicates the presence and severity of symptoms of neuropathy

204 [22]. Michigan Diabetic Neuropathy Score (MDNS) consists of 46-point score of clinical

205 neurological examination [22].

206

207 *2.5. Statistical analysis*

208 The sample size was determined through a priori power analysis for repeated measures

209 ANOVA using G*power (version 3.1.9.2). The calcualtion was based on a medium effect size

210 (= 0.06) for interaction between time and group, $\alpha = 0.05$, and power of 80% [26]. Based on a

211 previous study, the correlation between repeated measures was assumed to be 0.6 for nerve

212 conduction velocity [27]. According to these assumptions a sample size of 28 was required.

213 Considering a drop-out rate of 20%, the initial number of participants included in this study

214 was 34.

215 Data were expressed as mean ± SD. The Shapiro-Wilk test was used to confirm the

216 normal distribution of the data. The effects of exercise training on variables were determined

217 by repeated measures of ANOVA (Time x Group) and main effects of time were also reported.

218 For follow-up comparisons, Bonferroni's *post-hoc* analysis was carried out. An independent

219 samples *t*-test was also used to examine the change scores between groups over time (pre to

220 post-test). Effect sizes of the differences was indicated by partial *eta* squared. Pearson's

221 correlation coefficient was used to determine the association between variables. The

222 significance level was $P < 0.05$. Data were analyzed using SPSS for windows version 25 (SPSS

223 Inc, Chicago, III).

224

225 **3. Results**

226 A total of 34 participants were recruited to start the study and were randomized to two groups

227 (Figure 1). In total, 29 participants completed the study; 2 participants in the experimental

228 group and 3 participants in the control group failed to complete the study (Figure 1). The

229 participants' attendance to exercise sessions in the experimental group was 91-100%. The mean

230 and standard deviation for age, weight, fat percentage, and daily calorie intake are presented in

231 Table 1. There were no significant differences between groups at baseline (p > 0.05). Mean

232 and standard deviation for other variables are presented in Table 2. The baseline values were

233 similar in both groups (P > 0.05).

234   The supervised resistance training program was well tolerated with no adverse events

235 related to exercise program other than the expected transient muscle soreness during first few

236 sessions. The main intervention commenced with a familiarization period at lower intensity

237 and glucose level was strictly checked prior to exercise sessions, as previously described. As a

238 result, no adverse event including exercise-induced hypoglycemia/ hyperglycemia was

239 observed and the participants were able to deal with the procedure. Moreover, no adverse

240 events related to DSPN such as aggravated pain and foot ulceration were reported throughout

241 the study.

242   Data analysis indicated time x group interaction ($F = 4.996$, $p = 0.034$, $\eta_p^2 = 0.156$) and

243 main effect of time ($F = 8.725$, $p = 0.006$, $\eta_p^2 = 0.244$) for motor nerve action potential

244 amplitude (APA). Analysis of change scores between groups using independent samples t-test

245 also revealed a significant difference ($p = 0.034$). Follow-up comparison indicated an increase

246 of motor nerve APA in the experimental group (6% increase, $p = 0.048$). However, the time x

247 group interaction for sensory APA was not significant ($F = 2.329$, $p = 0.139$, $\eta_p^2 = 0.079$) and

248 analysis of change scores between groups using independent samples t-test also indicated no

249 significant difference ($p = 0.139$). A difference in motor NCV was observed with the time x

250 group interaction ($F = 13.456$, $p = 0.001$, $\eta_p^2 = 0.333$) and main effect of time ($F = 11.153$, $p =$

251 0.002, $\eta_p^2 = 0.292$), (Figure 2). Analysis of change scores between groups using independent

252 samples t-test also indicated a significant difference ($p = 0.001$). Follow-up comparison

253 indicated an increase of motor NCV in the experimental group (7% increase, $p = 0.016$). For

254 sensory NCV, there was also a time x group interaction ($F = 14.242$, $p = 0.001$, $\eta_p^2 = 0.345$)

255 and main effect of time ($F = 26.217$, $p = 0.0001$, $\eta_p^2 = 0.493$). Analysis of change scores

256 between groups using independent samples t-test also revealed a significant difference ($p =$

257 0.001). Follow-up comparison indicated an improvement of sensory NCV in the experimental

258 group (8% increase, $p = 0.002$). Furthermore, time x group interaction ($F = 12.541$, $p = 0.001$,

259 $\eta_p^2 = 0.317$) and main effect of time (F = 8.893, p = 0.006, $\eta_p^2 = 0.248$) were observed for

260 MDNS and analysis of change scores between groups using independent samples *t*-test also

261 revealed a significant difference (p = 0.001). Follow-up comparison revealed a reduction for

262 MDNS in the experimental group (10% reduction, p = 0.019). We also observed a time x group

263 interaction (F = 26.350, p = 0.0001, $\eta_p^2 = 0.494$) and main effect of time (F = 7.640, p = 0.010,

264 $\eta_p^2 = 0.221$) for MNSI (Figure 2). Analysis of change scores between groups using independent

265 samples *t*-test also revealed a significant difference (p = 0.0001) Follow-up comparison

266 indicated a marked reduction of MNSI in the experimental group (20% improvement, p =

267 0.003). Pearson's correlation coefficient analysis indicated the changes in MNSI score was

268 associated with the changes in HbA1C (r = 0.589, p = 0.021). The correlation of MNSI with

269 ABI and sensory nerve conduction did not reach significance (r = -0.447, p = 0.095 and r = -

270 0.473, p = 0.075, respectively).

271   A difference in CAVI was observed with the time x group interaction (F = 6.904, p =

272 0.014, $\eta_p^2 = 0.204$) and main effect of time (F = 12.703, p = 0.001, $\eta_p^2 = 0.320$), (Figure 2).

273 Analysis of change scores between groups using independent samples *t*-test also revealed a

274 significant difference (p = 0.001). Follow-up comparison revealed a reduction in the

275 experimental group (6.6% reduction, p = 0.039). For ABI, there was a time x group interaction

276 (F = 5.077, p = 0.033, $\eta_p^2 = 0.158$) but the main effect of time was not significant (F = 2.683,

277 p = 0.113, $\eta_p^2 = 0.090$). Analysis of change scores between groups using independent samples

278 *t*-test also revealed a significant difference (p = 0.033). Follow-up comparison did not show

279 significant alterations in the experimental group (4% increase, p = 0.123). A difference in ankle

280 systolic pressure was observed with the time x group interaction (F = 8.670, p = 0.007, $\eta_p^2 =$

281 0.243) and main effect of time (F = 4.258, p = 0.049, $\eta_p^2 = 0.136$). Analysis of change scores

282 between groups using independent samples *t*-test also revealed a significant difference (p =

283 0.007). Follow-up comparison revealed an increase in the experimental group (3.4% increase,

284 $p = 0.001$). For brachial systolic pressure, there was neither significant time x group interaction
285 ($F = 0.867$, $p = 0.360$, $\eta_p^2 = 0.031$) nor the main effect of time ($F = 1.000$, $p = 0.326$, $\eta_p^2 =$
286 0.036).

287 We also observed a time x group interaction ($F = 11.240$, $p = 0.002$, $\eta_p^2 = 0.294$) and
288 main effect of time ($F = 5.712$, $p = 0.024$, $\eta_p^2 = 0.175$) for HbA1C (Figure 2). Analysis of
289 change scores between groups using independent samples *t*-test also revealed a significant
290 difference ($p = 0.002$). Follow-up comparison revealed a reduction in HbA1C in the
291 experimental group (10% reduction, $p = 0.025$).

292

293 **4. Discussion**

294 The aim of the study was to investigate the effect of resistance training on symptoms of
295 neuropathy, nerve conduction and arterial stiffness in older adults with DSPN. For the first
296 time, we observed that low-to-moderate intensity resistance training improved measurements
297 of DSPN including neuropathic symptoms, nerve conduction velocity, nerve action potential
298 amplitude, measurements of arterial stiffness including CAVI and ABI, and HbA1c levels in
299 older adults with DSPN. Besides, no major complications and adverse events directly related
300 to exercise training were reported throughout the intervention. Accordingly, low-to-moderate
301 intensity resistance training can be an effective and safe therapeutic intervention for this group
302 of patients.

303 Nerve conduction velocity and nerve APA improved following 12-week resistance
304 exercise regimen in older adults with DSPN. This finding is partly in accordance with some
305 previous studies that reported enhanced NCV of sensory and motor nerves following aerobic
306 exercise training [26, 27]. The evidence showed that the neural system responds positively to
307 physical activity through morphological and functional adaptations to the training stimulus [6,
308 28-30]. Exercise training has been shown to decrease apoptosis of Schwann cells and increase

309 myelin thickening [6], increase Schwann cell proliferation [28], enhance axonal regeneration

310 of the peroneal nerve [30] and improve axon transport and electrophysiological properties [29].

311 Resistance training also enhances neural activation and neuroplasticity that occurs at earlier

312 stages of training program. These changes likely explain the initial functional adaptations and

313 subsequent strength gains following resistance training program [31]. Moreover, resistance

314 exercise can activate the effects of neurotrophin because it can increase brain-derived

315 neurotrophic factor (BDNF) expression and its receptor tropomyosin receptor kinase B (trkB)

316 [32]. BDNF and its receptor seem to be substantial modulator for axon regeneration as the

317 continuous presence of BDNF enhanced axon regeneration after nerve damage [33].

318   The improvements in the measurements of nerve conduction in the current study might

319 be partly explained by local and direct impact of resistance training on peripheral nerves.

320 Morphological changes in peripheral nerves are not the only factor to explain nerve conduction

321 improvements [34]; functional alterations are also essential so that improved neural function

322 can be attributed to the reinforcement of existing sensory-motor pathways rather than

323 generating additional pathways [35]. In the present study, the changes in NCV and APA were

324 coupled with improved neuropathy symptoms. Another established effect of exercise,

325 irrespective of type, is hypoalgesia induced by exercise [34]. Groover et al, (2013) indicated

326 that exercise can regulate cutaneous sensation and nociceptive threshold related to prediabetes

327 [36]. It may be explained by cutaneous regeneration as exercise training has been shown to

328 increase distal leg intraepidermal nerve fiber density (IENFD) in diabetic patients with [37]

329 and without neuropathy [38]. Although IENDF is a measure of integrity of small diameter

330 axons and evaluates unmyelinated axons, it is correlated with large myelinated fiber surrogates

331 such as nerve conduction studies [38]. Singleton et al, (2014) indicated that development of

332 abnormal neurological examinations was remarkably reduced in patients receiving exercise

333 intervention [38]. Additionally, exercise training increased the endogenous opioid production

which might play a role in neuropathic pain reversal induced by exercise [39]. These alterations suggest that exercise training provides benefits to both central and peripheral nervous system. These exercise effects are valuable for this population and we contend that the low-to-moderate intensity resistance exercise program in this study also has the potential to improve neural function in older adults with DSPN, which is manifested by reduced neuropathy symptoms.

Besides neural alterations, glucose regulation and vascular function are essential contributors of nerve function in DSPN. Hyperglycemia is the principal determinant of the development of DSPN. It has been suggested that 1% increase in HbA1C is associated with almost 10-15% increase in frequency of DSPN [40]. Constant or fluctuating hyperglycemia can induce sorbitol accumulation in nerves, oxidative stress, chronic inflammation and endothelial dysfunction [2] and lead to peripheral nerve damage in type 2 diabetes. Hence, aggressive control of glucose is crucial for type 2 diabetes and related complications, especially DSPN. Optimized glucose control reduces nerve conduction abnormalities and is important in the prevention and treatment of DSPN [41]. We observed a marked reduction in HbA1C from 9.09% to 8.11% following a 12 week resistance exercise program that was correlated with alterations in neuropathy symptoms, which supported previous work showing that improvement in signs of DSPN were coupled with a reduction of HbA1C [37]. The effect of different modes of exercise on glucose regulation is well documented [15] and resistance training has been suggested to be as effective as aerobic training to reduce levels of HbA1C [15]. One study has shown that resistance training was superior at reducing HbA1C level to aerobic training [13]. Hence, the application of resistance training can be clinically significant because of the HbA1C lowering effects, the potential to lower the prevalence and progression of DSPN [31].

DSPN has been shown to be significantly associated with blood flow to the peripheral nerves and arterial stiffness [3, 42]. Increased arterial stiffness in the lower extremity can

359 compromise blood flow to foot arteries [3]; this has been shown with femoral arterial stiffness

360 in diabetes patients being closely related with ischemic symptoms of the lower extremity.

361 Reduced endoneural blood flow causes hypoxia in the peripheral nerves that can influence

362 nerve conduction [2]. For instance, NCV is shown to be sensitive to blood supply, and so the

363 restoration of blood flow to the lower extremity can enhance conduction velocity of peroneal

364 nerve in patients with type 2 diabetes [12]. This study showed a reduction in the mean values

365 of CAVI and an increase in ABI following the 12-week resistance training intervention. A

366 recent systematic review concluded that resistance training at lower intensities could bring

367 about cardiovascular health benefits and may improve measures of arterial stiffness [42]. CAVI

368 reflects heart-to-ankle stiffness of arterial walls and ABI is the ankle-brachial pressure index

369 reflecting the degree of arterial blockage in the lower limbs that is reduced in peripheral arterial

370 diseases. Despite the non-significant alterations in brachial systolic pressure, ankle pressure

371 did improve following resistance training. Thus, improvements in ABI are thought to be driven

372 by an improvement in ankle systolic pressure rather than changes in brachial systolic pressure.

373 A potential explanation for improved ankle pressure and ABI by resistance training might be

374 improved blood flow in the lower extremities [43]. Exercise training enhances endothelium-

375 dependent and independent vasodilation, nitric oxide synthesis and bioavailability and

376 expression of growth factors that can lead to improved blood flow to the limbs [44]. Even

377 though leg vascular function can be precisely assessed via ultrasound or femoral-ankle pulse

378 wave velocity, ABI has also a well-supported prognostic value that reflects improved leg

379 vascular function following resistance training in this study. Regarding this, Fahs et al. (2011)

380 reported that acute resistance exercise increased arterial compliance and calf blood flow. They

381 found that high-intensity resistance exercise at 70% of 1RM caused greater elevation of calf

382 vascular conductance, elasticity of large artery and small artery compared to low intensity

383 exercise at 20% of 1RM [14]. Hence, high-intensity resistance exercise could be more effective

than low-intensity exercise to improve large and small artery blood flow. The exercise intensity in our study roughly corresponded to the high-intensity protocol implemented by Fahs et al. (2011). Muscle contraction during resistance exercise causes hyperemia that can immediately increase arterial compliance and blood perfusion to the active sites. Over time, with repeated exposure, exercise-induced elevation of blood flow can increase blood supply to the vasa nervorum and therefore contribute to restoring nerve function [46]. Besides, resistance exercise reduces systemic vascular resistance indicating relaxation of vascular smooth muscle. This can further explain improved arterial compliance and blood flow by resistance exercise training [14]. Hence, improved measurements of nerve conduction in this study could also be attributed to factors other than glucose regulation alone i.e., restoration of blood flow and oxygen supply to peripheral nerves as a result of resistance training. However, due to little understandings in this area it needs to be clarified in future studies.

In contrast, some previous studies did not report the effectiveness of an aerobic exercise program on some measurements of nerve conduction in DSPN [25, 37]. Dixit et al, (2014) reported that aerobic training over 8 weeks had no significant effect on nerve APA of the peroneal nerve in patients with DSPN [26]. This discrepancy might be explained by the exercise type (aerobic versus resistant-type training), the intensity and duration and hence determine nerve response to training. Exercise has been suggested to be an effective treatment for nerve functional recovery, yet the effects depend on the type, duration and intensity of the protocol [17]. Another possible mediator in the response to exercise is sex; where a previous animal study reported that the effect of different exercises on axon regeneration after peripheral nerve injury is sex-dependent [47]. This study showed that male animals were more responsive to continuous exercise while female animals responded well to interval exercise [47]. This observation might go some way to explaining the differences in studies where a male-only cohort was used. Based on the contribution of sex on nerve responsiveness to exercise [47], the

409 mediatory effects of resistance training to this intervention could be explored in future studies

410 can extend the understandings in this area.

411

412 **5. Limitations and Future Directions**

413 The study includes some strength and limitations to be acknowledged. In spite of several

414 medical concerns with this group of patients, the adherence to exercise intervention was very

415 good and drop-out rate was trivial. Furthermore, walking and jogging is the frequently used

416 and prescribed form of exercise in this population. This is the first randomized trial that

417 indicates feasibility of resistance-type exercise program for older adults with DSPN. The

418 homogenous group of male participants show the benefits of resistance training for this cohort.

419 However, the data might be limited to this cohort and further studies on female participants

420 should be conducted to increase our understanding regarding effect of sex on exercise-induced

421 neural changes in DSPN and extend the generalizability of the findings.

422 We observed benefits of resistance training on neuropathic symptoms in older male

423 adults with DSPN. Since diabetes-related nerve damage is associated with impaired balance

424 and increased risk of falling [48], further research is required to determined how the effects of

425 resistance training can translate into functional measures such as daily physical activities and

426 quality of life. A greater understanding of the effects of resistance-type exercise on functional

427 measurements can provide further implications for exercise recommendations to this

428 population. One might assume that improved nerve conduction measurements and symptoms

429 of neuropathy can be resulted from the concurrent effect of resistance training on metabolic

430 and vascular measures in addition to direct local effects on peripheral nerves. However, due to

431 the lack of understandings in this area, this should be explored in more detail with mechanistic

432 studies.

433

6. Conclusions/ Implications

This study provide new data that older patients with DSPN respond positively to low-to-moderate intensity resistance training by improved neuropathy symptoms, nerve conduction, arterial stiffness and glucose regulation. What is important to note is that resistance-type training is a safe and feasible intervention to improve the signs and symptoms associated with the progression of DSPN and hence can be used as an effective intervention to improve these variables. These new data can be used as a platform for the exploration of other benefits of this type of intervention, but critically, can be applied in patients with type 2 diabetes and specifically to the growing population of older adults with diabetic peripheral neuropathy.

8. Authors' Contribution

The study was conceptualized and designed by FG; data were collected by FG, RK and BM; data were interpreted by FG and GH; and manuscript preparation was undertaken by FG and GH. The final version of the manuscript was approved by all authors.

9. Competing interests

The authors have no conflict of interest to declare.

References

1. Tesfaye S, Boulton AJ, Dyck PJ, et al. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. *Diabetes Care*. 2010;33(10):2285-93. Doi: 10.2337/dc10-1303.

2. Cameron NE, Eaton SE, Cotter MA, et al. Vascular factors and metabolic interactions in the pathogenesis of diabetic neuropathy. *Diabetologia.* 2001;44(11):1973-88. Doi: 10.1007/s001250100001.

3. Suzuki E, Kashiwagi A, Nishio Y, et al. Increased arterial wall stiffness limits flow volume in the lower extremities in type 2 diabetic patients. *Diabetes Care*. 2001;24(12):2107-14. Doi: 10.2337/diacare.24.12.2107.

4. Vinik AI, Strotmeyer ES, Nakave AA, et al. Diabetic neuropathy in older adults. *Clin Geriatr Med*. 2008;24(3):407-35. DOI: 10.1016/j.cger.2008.03.011.

5. Melcangi RC, Magnaghi V, Martini L. Aging in peripheral nerves: regulation of myelin protein genes by steroid hormones. *Prog Neurobiol*. 2000;60(3):291-308. Doi: 10.1016/s0301-0082(99)00028-3.

6. Shokouhi G, Tubbs RS, Shoja MM, et al. The effects of aerobic exercise training on the age-related lipid peroxidation, Schwann cell apoptosis and ultrastructural changes in the sciatic nerve of rats. *Life Sci*. 2008;82(15-16):840-6. Doi: 10.1016/j.lfs.2008.01.018.

7. Wang YJ, Zhou CJ, Shi Q, et al. Aging delays the regeneration process following sciatic nerve injury in rats. *J Neurotrauma*. 2007;24(5):885-94. Doi: 10.1089/neu.2006.0156.

8. Patel KV, Phelan EA, Leveille SG, et al. High prevalence of falls, fear of falling, and impaired balance in older adults with pain in the United States: findings from the 2011 National Health and Aging Trends Study. *J Am Geriatr Soc*. 2014;62(10):1844-52. Doi: 10.1111/jgs.13072.

483 9. Perkins BA, Olaleye D, Zinman B, et al. Simple screening tests for peripheral
484 neuropathy in the diabetes clinic. *Diabetes Care*. 2001;24(2):250-6. DOI:
485 10.2337/diacare.24.2.250.

486 10. Schreiber AK, Nones CF, Reis RC, et al. Diabetic neuropathic pain: physiopathology
487 and treatment. *World J Diabetes*. 2015;6(3):432. Doi: 10.4239/wjd.v6.i3.432.

488 11. Ang L, Jaiswal M, Martin C, et al. Glucose control and diabetic neuropathy: lessons
489 from recent large clinical trials. *Curr Diab Rep*. 2014;14(9):528. DOI: 10.1007/s11892-014-
490 0528-7

491 12. Young MJ, Veves A, Smith JV, et al. Restoring lower limb blood flow improves
492 conduction velocity in diabetic patients. *Diabetologia*. 1995;38(9):1051-4. Doi:
493 10.1007/BF00402174.

494 13. Cauza E, Hanusch-Enserer U, Strasser B, et al. The relative benefits of endurance and
495 strength training on the metabolic factors and muscle function of people with type 2 diabetes
496 mellitus. *Arch Phys Med Rehabil*. 2005;86(8):1527-33. Doi: 10.1016/j.apmr.2005.01.007.

497 14. Fahs CA, Rossow LM, Seo DI, et al. Effect of different types of resistance exercise on
498 arterial compliance and calf blood flow. *Eur J Appl Physiol*. 2011;111(12):2969-75. Doi:
499 10.1007/s00421-011-1927-y.

500 15. Gordon BA, Benson AC, Bird SR, et al. Resistance training improves metabolic health
501 in type 2 diabetes: a systematic review. *Diabetes Res Clin Prac*t. 2009;83(2):157-75. Doi:
502 10.1016/j.diabres.2008.11.024.

503 16. Krause Neto W, Silva WdA, Ciena AP, et al. Effects of strength training and anabolic
504 steroid in the peripheral nerve and skeletal muscle morphology of aged rats. *Front Aging*
505 *Neurosci*. 2017;9:205. DOI: 10.3389/fnagi.2017.00205.

506 17. Udina E, Cobianchi S, Allodi I, et al. Effects of activity-dependent strategies on

507 regeneration and plasticity after peripheral nerve injuries. *Ann Anat*. 2011;193(4):347-53. Doi:

508 10.1016/j.aanat.2011.02.012.

509 18. Molteni R, Zheng JQ, Ying Z, et al. Voluntary exercise increases axonal regeneration

510 from sensory neurons. *Proc Natl Acad Sci U S A*. 2004;101(22):8473-8. Doi:

511 10.1073/pnas.0401443101.

512 19. Streckmann F, Zopf EM, Lehmann HC, et al. Exercise intervention studies in patients

513 with peripheral neuropathy: a systematic review. *Sports Med*. 2014;44(9):1289-304. Doi:

514 10.1007/s40279-014-0207-5.

515 20. Mueller MJ, Tuttle LJ, LeMaster JW, et al. Weight-bearing versus nonweight-bearing

516 exercise for persons with diabetes and peripheral neuropathy: a randomized controlled trial.

517 *Arch Phys Med Rehabil*. 2013;94(5):829-38. Doi: 10.1016/j.apmr.2012.12.015.

518 21. Kluding PM, Bareiss SK, Hastings M, et al. Physical training and activity in people

519 with diabetic peripheral neuropathy: paradigm shift. *Phys Ther*. 2017;97(1):31-43. DOI:

520 10.2522/ptj.20160124.

521 22. Feldman EL, Stevens M, Thomas PK, et al. A practical two-step quantitative clinical

522 and electrophysiological assessment for the diagnosis and staging of diabetic neuropathy.

523 *Diabetes Care*. 1994;17(11):1281-9. DOI: 10.2337/diacare.17.11.1281.

524 23. Dyck PJ, Albers JW, Anderson H, et al. Diabetic polyneuropathies: update on research

525 definition, diagnostic criteria and estimation of severity. Diabetes Metab Res Rev. 2011; 27(7):

526 620-628. DOI: 10.1002/dmrr.1226.

527 24. Sheri RC, Sigal RJ, Fernhall B, et al. Exercise and Type 2 Diabetes: The American College

528 of Sports Medicine and the American Diabetes Association: joint position statement. *Diabetes*

529 *Care*. 2010; 33(12):147-67. DOI: 10.2337/dc10-9990.

530   25.   Kubozono T, Miyata M, Ueyama K, et al. Clinical significance and reproducibility of

531   new arterial distensibility index. *Circ J*. 2007;71(1):89-94. Doi: <u>10.1253/circj.71.89.</u>

532   26.   Dixit S, Maiya AG, Shastry BA. Effect of aerobic exercise on peripheral nerve

533   functions of population with diabetic peripheral neuropathy in type 2 diabetes: a single blind,

534   parallel group randomized controlled trial. *J Diabetes Complications*. 2014;28(3):332-9. Doi:

535   <u>10.1016/j.jdiacomp.2013.12.006</u>.

536   27.   Gholami F, Nikookheslat S, Salekzamani Y, et al. Effect of aerobic training on nerve

537   conduction in men with type 2 diabetes and peripheral neuropathy: A randomized controlled

538   trial. *Neurophysiol Clin*. 2018;48(4):195-202. Doi: <u>10.1016/j.neucli.2018.03.001</u>.

539   28.   Bobinski F, Martins DF, Bratti T, et al. Neuroprotective and neuroregenerative effects

540   of low-intensity aerobic exercise on sciatic nerve crush injury in mice. *Neuroscience*.

541   2011;194:337-48. Doi: <u>10.1016/j.neuroscience.2011.07.075.</u>

542   29.   Gardiner P, Dai Y, Heckman CJ. Effects of exercise training on α-motoneurons. *J Appl*

543   *Physiol*. 2006;101(4):1228-36. Doi: <u>10.1152/japplphysiol.00482.2006</u>.

544   30.   Sabatier MJ, Redmon N, Schwartz G, et al. Treadmill training promotes axon

545   regeneration in injured peripheral nerves. *Exp Neurol*. 2008;211(2):489-93. Doi:

546   <u>10.1016/j.expneurol.2008.02.013</u>.

547   31.   Hortobágyi T, Granacher U, Fernandez-Del-Olmo M, et al. Functional relevance of

548   resistance training-induced neuroplasticity in health and disease. *Neurosci Biobehav Rev*.

549   2021:122:79-91. DOI: 10.1016/j.neubiorev.2020.12.019.

550   32.   Yarrow JF, White LJ, McCoy SC, et al. Training augments resistance exercise induced

551   elevation of circulating brain derived neurotrophic factor (BDNF). *Neurosci Lett.*

552   2010;479(2):161-5. Doi: <u>10.1016/j.neulet.2010.05.058</u>.

33. Knaepen K, Goekint M, Heyman EM, et al. Neuroplasticity—exercise-induced response of peripheral brain-derived neurotrophic factor. *Sports Med.* 2010;40(9):765-801. Doi: 10.2165/11534530-000000000-00000.

34. Cooper MA, Kluding PM, Wright DE. Emerging relationships between exercise, sensory nerves, and neuropathic pain. *Fron Neurosci*. 2016;10:372. Doi: 10.3389/fnins.2016.00372.

35. De Leon RD, Hodgson JA, Roy RR, et al. Locomotor capacity attributable to step training versus spontaneous recovery after spinalization in adult cats. *J Neurophysiol.* 1998;79(3):1329-40. Doi: 10.1152/jn.1998.79.3.1329.

36. Groover AL, Ryals JM, Guilford BL, et al. Exercise-mediated improvements in painful neuropathy associated with prediabetes in mice. *Pain*. 2013;154(12):2658-67. Doi: 10.1016/j.pain.2013.07.052.

37. Kluding PM, Pasnoor M, Singh R, et al. The effect of exercise on neuropathic symptoms, nerve function, and cutaneous innervation in people with diabetic peripheral neuropathy. *J Diabetes Complications*. 2012;26(5):424-9. Doi: 10.1016/j.jdiacomp.2012.05.007.

38. Singleton JR, Marcus RL, Jackson JE, et al. Exercise increases cutaneous nerve density in diabetic patients without neuropathy. *Ann Clin Transl Neurol*. 2014;1(10)-844-9. DOI: 10.1002/acn3.125.

38. Stagg NJ, Mata HP, Ibrahim MM, et al. Regular exercise reverses sensory hypersensitivity in a rat neuropathic pain modelrole of endogenous opioids. *Anesthesiology*. 2011;114(4):940-8. Doi: 10.1097/ALN.0b013e318210f880.

40. Lai YR, Chiu WC, Huang CC, et al. HbA1C variability is strongly associated with the severity of peripheral neuropathy in patients with type 2 diabetes. *Front Neurosci*. 2019;13:90. Doi: 10.3389/fnins.2019.00090.

577   41.   Callaghan BC, Little AA, Feldman EL, et al. Enhanced glucose control for preventing

578   and treating diabetic neuropathy. *Cochrane Database Syst Rev*. 2012;6(6);CD007543. Doi:

579   <u>10.1002/14651858.CD007543.pub2</u>.

580   42.   García-Mateo P, García-de-Alcaraz A, Rodríguez-Perez M, et al. Effect of resistance

581   training on arterial stiffness in healthy people : A systematic review. *J Sports Sci Med*.

582   2020;19(3):444-451. PMID:<u> 32874096.</u>

583   43.   Gibbs BB, Dobrosielski DA, Althouse AD, et al. The effect of exercise training on

584   ankle-brachial index in type 2 diabetes. *Atherosclerosis*. 2013;230(1):125-130.

585   44.   Kim ES, Moon SD, Kim HS, et al. Diabetic peripheral neuropathy is associated with

586   increased arterial stiffness without changes in carotid intima–media thickness in type 2

587   diabetes. *Diabetes Care*. 2011;34(6):1403-5. Doi: <u>10.2337/dc10-2222</u>.

588   45.   Lee JH, Lee R, Hwang MH, et al. The effects of exercise on vascular endothelial

589   function in type 2 diabetes: a systematic review and meta-analysis. *Diabetol Metab Syndr*.

590   2018;10(1):15. Doi:<u> 10.1186/s13098-018-0316-7</u>.

591   46.   Tesfaye S, Harris ND, Wilson RM, et al. Exercise-induced conduction velocity

592   increment: a marker of impaired peripheral nerve blood flow in diabetic neuropathy.

593   *Diabetologia*. 1992;35(2):155-9. Doi: <u>10.1007/BF00402548</u>.

594   47.   Wood K, Wilhelm JC, Sabatier MJ, et al. Sex differences in the effectiveness of

595   treadmill training in enhancing axon regeneration in injured peripheral nerves. *Dev Neurobiol*.

596   2012;72(5):688-98. Doi: 10.1002/dneu.20960.

597   48.   Brown SJ, Handsaker JC, Bowling FL, et al. Diabetic peripheral neuropathy

598   compromises balance during daily activities. *Diabetes Care*. 2015;38(6):1116-22. Doi:

599   <u>10.2337/dc14-1982</u>.

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Table 1. Mean ± SD values for age, height, weight, fat percentage, calorie intake, and selected lower and upper-body strength.

Group Variable	Experimental (n=15)		Control (n=14)	
	Pre	**Post**	**Pre**	**Post**
Age (years)	63 ± 3	-	64 ± 3	-
Height (cm)	170.2 ± 5.1	-	168.7 ± 4.0	-
Weight (kg)	74.9±9.0	73.5±9.0	72.6±8.9	73.2±8.6
Fat (%)	27.7±4.4	26.6±4.5	26.6±5.1	27.0±4.8
Calorie intake (Kcal)	2199±267	2216±255	2272±263	2308±221
Squat 1RM (kg)	56.7±11.0	65.4±9.7	-	-
Bench press 1RM (kg)	43.0±8.3	49.4±7.4	-	-

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634 **Table 2. Description of the changes in mean and standard deviation for variables at baseline and following**

635 **12 weeks in groups.**

Group	Experimental (Mean ± SD, 95% CI) (n=15)		Control (Mean ± SD, 95% CI) (n=14)		P	EF
	Pre	**Post**	**Pre**	**Post**		
HbA1C (%)	9.09±1.82 (8.08–10.10)	8.11±1.45 (7.31–8.33)	9.97±1.82 (8.91–11.02)	10.13±1.50 (9.26–11.00)	0.002	0.294
HbA1C (mmol/ mol)	75.86±19.79(64.90-86.82)	65.13±15.72(56.42-73.4)	85.28±19.61(73.95-96.61)	87.29±16.55(77.72-96.84)	0.002	0.309
CAVI	8.51±0.73 (8.10–8.92)	7.94±0.69 (7.56–8.34)	8.30±0.52 (7.99–8.60)	8.21±0.48 (7.93–8.49)	0.014	0.204
ABI	0.94±0.12 (0.87–1.01)	0.98±0.10 (0.92–1.04)	0.95±0.08 (0.90–0.99)	0.94±0.08 (0.89–0.99)	0.033	0.158
BSP	137.60±10.90(131.55–143.64)	135.80±8.28(131.21–140.28)	138.35±9.06(133.12–143.59)	138.28±6.46(134.55–142.01)	0.360	0.031
ASP	128.86±9.90(123.38–134.35)	133.33±9.64(127.99–138.67)	131.57±9.04(126.34–136.79)	130.78±9.25(125.44–136.13)	0.007	0.243
Sensory APA (µV)	4.54±2.06 (3.49–5.69)	4.63±2.05 (3.50–5.77)	4.67±2.01 (3.51–5.83)	4.64±1.99 (3.48–5.79)	0.139	0.079
Motor APA (mV)	2.92±1.16 (2.27–3.57)	3.12±1.15 (2.48–3.76)	2.95±1.33 (2.18–3.72)	2.98±1.29 (2.23–3.73)	0.034	0.156
Sensory NCV (m/s)	27.62±8.75 (22.77-32.47)	30.06±8.56 (25.31–34.80)	28.56±8.92 (23.41–33.71)	28.93±8.69 (23.91–33.95)	0.001	0.345
Motor NCV (m/s)	33.01±8.88 (28.09–37.92)	35.38±8.72 (30.55–40.21)	36.02±9.41 (30.58–41.45)	35.90±8.97 (30.72–41.09)	0.001	0.333
MDNS	16.53±6.18 (13.11–19.96)	14.87±5.95 (11.57–18.16)	15.36±5.73 (12.05–18.67)	15.50±5.58 (12.28–18.72)	0.001	0.317
MNSI	8.26±1.94 (7.18 – 9.34)	6.60±1.40 (5.82-7.37)	7.50±2.06 (6.30-8.69)	8.00±1.79 (7.01-8.98)	0.0001	0.494

636 Abbreviations: EF, Effect size; CAVI, Cardio-ankle vascular index; ABI, Ankle-brachial index; BSP, Brachial

637 systolic pressure; ASP, Ankle systolic pressure; APA, Action potential amplitude; NCV, Nerve conduction

638 velocity; MDNS, Michigan diabetic neuropathy score; MNSI, Michigan neuropathy screening instrument.

639 P values are presented for Time x Group interactions.

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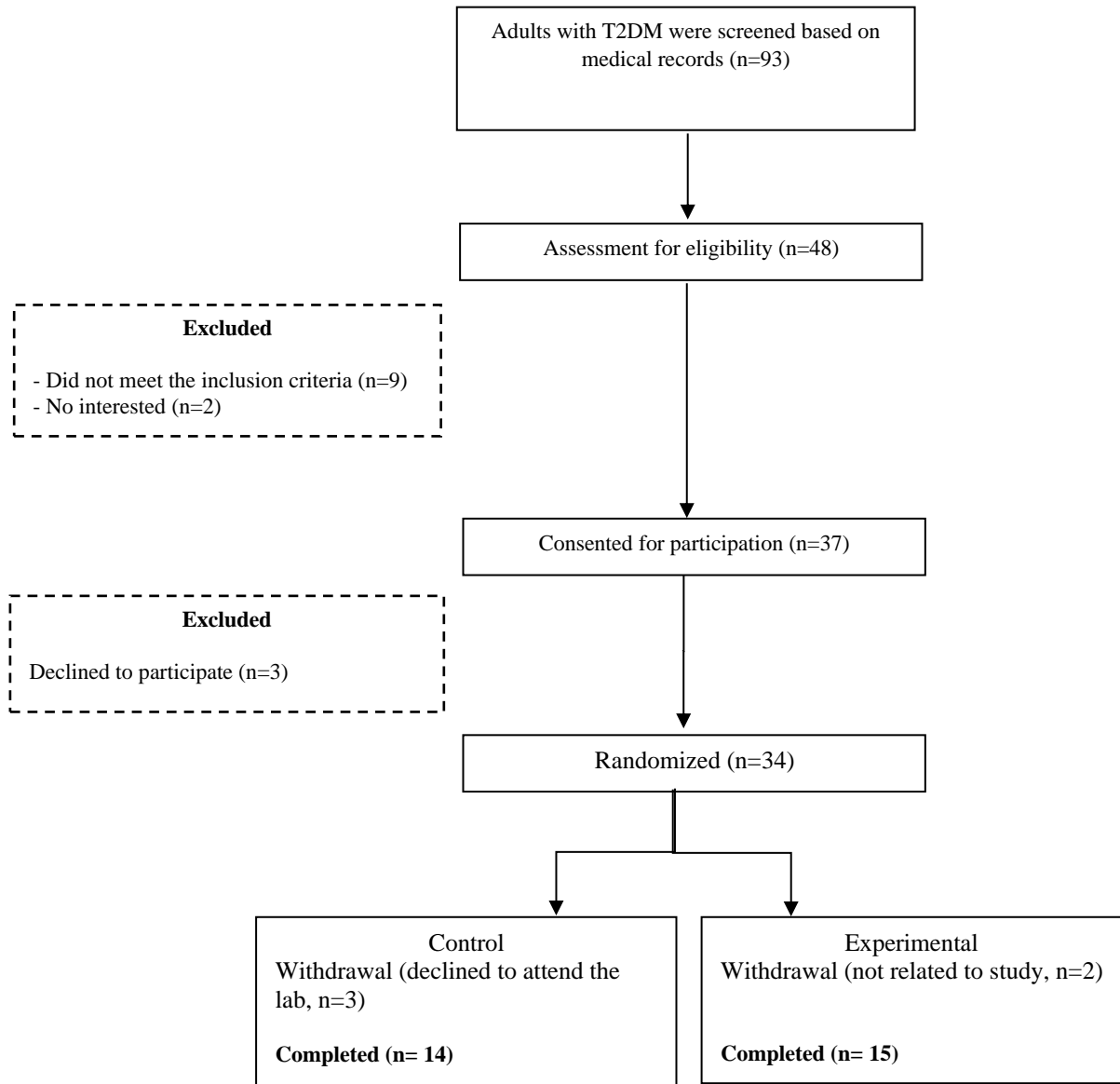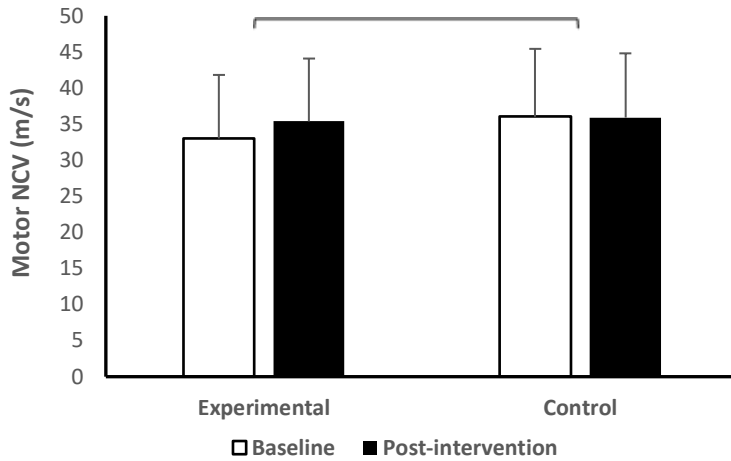676
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Adults with T2DM were screened based on medical records (n=93)

Assessment for eligibility (n=48)

Excluded

- Did not meet the inclusion criteria (n=9)
- No interested (n=2)

Consented for participation (n=37)

Excluded

Declined to participate (n=3)

Randomized (n=34)

Control
Withdrawal (declined to attend the lab, n=3)

Completed (n= 14)

Experimental
Withdrawal (not related to study, n=2)

Completed (n= 15)

Figure 1. Consort flow chart of participant enrolment, randomization and analysis

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Figure 2. Changes in A) Motor Nerve Conduction Velocity (NCV); HbA1C; C) Cardio-Ankle
Vascular Index (CAVI); and D) Michigan Diabetic Neuropathy Instrument (MNSI) score from
pre to post-test. The data are presented as Mean ± SD. * Significant difference with
corresponding pre-test values $P < 0.05$. [#] Significant Time x Group interaction at $P < 0.05$.
Experimental ▨ Control ▢