

Encouraging effects of a short-term, adapted Nordic diet intervention on skin microvascular function and skin oxygen tension in younger and older adults

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Title: Encouraging effects of a short-term, adapted Nordic diet intervention on skin microvascular function and skin oxygen tension in younger and older adults

Running Head: Adapted Nordic diet and microvascular function

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Highlights

- 39 • Effects of a 4-week adapted Nordic diet on microvascular function in younger and
40 older adults were assessed
- 41 • Laser Doppler Flowmetry measured cutaneous microvascular functioning
- 42 • Transcutaneous Oxygen monitoring measured skin oxygen tension
- 43 • Health markers were investigated concurrently
- 44 • Microvascular health, body-fat % and peak heart rate during exercise were improved
45 followed the diet.

Abstract

Objective: Microvascular benefits of regional diets are appearing in the literature however little is known about Nordic-type diets. We investigated the effects of short-term adapted Nordic diet on microvascular function in younger and older individuals at rest and during activity.

Research Method & Procedures: Thirteen young [Mean: 28, SD: (5)] and fifteen older participants [Mean: 68, SD: (6)] consumed a modified Nordic diet for four weeks. Laser Doppler Flowmetry and Transcutaneous oxygen monitoring assessed cutaneous microvascular function and oxygen tension pre and post-intervention; blood pressure, body mass, body-fat%, ratings of perceived exertion and peak heart rate during activity were examined concurrently.

Results: Axon-mediated vasodilation improved in older participants [1.17 (0.30) to 1.30 (0.30); $P < 0.05$]. Improvements in endothelium-dependent vasodilation were noted in young [1.67 (0.50) to 2.03 (0.62); $P < 0.05$] and older participants [1.49 (0.37) to 1.63 (0.39); $P < 0.05$]. Reduced peak heart rate during activity was noted in older participants only [36.5(8.9) to 35.3(8.5); $P < 0.05$] and reduced body-fat % in young participants only [young = 27.2 (8.3) to 25.2 (8.8); $P < 0.05$]. No other variables reached statistical significance however trends were observed.

Conclusions: We observed statistically-significant improvements in microvascular function, peak heart rate and body composition. Following an adapted Nordic diet might improve microvascular health.

Keywords

Nordic Diet; Laser Doppler Flowmetry; Oxygen Tension

Cardiovascular disease (CVD) is the number one cause of death worldwide with 17.5 million deaths reported in 2012 (WHO, 2016). Risk factors for developing CVD include inflammatory diseases such as type II diabetes and hypertension; aging, gender and lifestyle factors such as smoking and poor nutrition (WHO 2016). Endothelial dysfunction, a pathological condition characterised by impaired vasodilation and systemic inflammation (Hadi et al. 2007), is a precursor of acute coronary syndromes, atherosclerosis and CVD (Deanfield et al. 2007). Endothelial dysfunction however appears to be reversible and endothelial health can be improved by modifying cardiovascular risk factors (Hadi et al. 2005). Emerging literature has therefore sought to investigate the effects of lifestyle modifications as possible treatment strategies (Klonizakis et al. 2013) and dietary intervention is one lifestyle modification that appears to be promising (Nordman et al. 2011).

Dietary interventions, however, are difficult to sustain, and factors such as taste preferences, culinary habits and social acceptability might contribute to poor long-term adherence (Poulsen et al. 2015). Bere and Brug (2009) recommend that strategies tailored to regional eating preferences might lead to better long-term success, and interestingly, data is beginning to suggest that regional diets might offer health benefits. Indeed, evidence now suggests that the Mediterranean diet can reduce CVD* risk (Nordmann et al. 2011), alleviate metabolic syndrome (Kastorini et al. 2011), reduce blood pressure and enhance weight loss (Esposito et al. 2011).

The Nordic diet is a regional diet that encourages the consumption of Nordic vegetables and fruits as well as whole grains, fish, rapeseed oil and low-fat dairy products. Early data

* **Abbreviations:** CVD, Cardiovascular Disease; NND, New Nordic Diet; LDF, Laser Doppler Flowmetry; TcPO₂: Transcutaneous Oxygen monitoring; BMI, Body Mass Index; CVC, Cutaneous Vascular Conductance; RPE, Rating of Perceived Exertion; SD, Standard Deviation; NO, Nitric Oxide; MD, Mediterranean Diet; PUFA, Polyunsaturated Fatty Acids

suggests that this diet might lead to reduced inflammation (Kanerva et al. 2014a), improved insulin metabolism (De Mello et al. 2011) and weight loss (Poulsen et al. 2015). Cardiovascular-health benefits of the diet are also now beginning to appear in the literature: Adamsson et al. (2011) demonstrated that a 10-week intervention led to lower cholesterol, reduced blood pressure and decreased serum insulin in hypercholesterolaemic participants. To date, however, microvascular health effects of Nordic diets have yet to be explored. The integrity of the microcirculation to sustain blood flow, tissue oxygenation and nutrient delivery affects susceptibility to disease, and appears to decline with age (Tew et al. 2010). Identifying strategies that maintain or improve microvascular function are therefore important for sustaining long-term health.

The aim of this study was to investigate the effects of a short-term, adapted Nordic diet (AND), modified for British taste preferences, on the microvasculature, by assessing tissue oxygenation and endothelial function. The circulatory system functions differently at rest and during activity (Abraham et al. 2003) and age-related endothelial dysfunction, characterised by diminished arterial vasodilation and reduced nitric oxide supply, has been observed in older adults (Gates et al. 2009). We therefore compared the effects of the diet in younger (18-35 years old) and older sedentary participants (55-75 years old) at rest and during sub-maximal exercise. We hypothesised that the intervention would improve microvascular health and endothelial function in both groups, with older participants experiencing greater improvements.

Material and Methods

Ethical Approval

Ethical approval for this research was granted by the Sheffield Hallam University's Health and Wellbeing Research Ethics Committee. This research was conducted in accordance with the Declaration of Helsinki.

Participants

Sixteen young participants aged 18-35 years [$M = 28(5)$] and sixteen older participants aged 55-75 years [$M = 64(6)$] provided informed consent. Recruitment took place via posters, word of mouth and through the emailing systems of Sheffield Hallam University and the University of Sheffield. Participants' eligibility was assessed pre-intervention using physical activity and nutrition questionnaires. The long International Physical Activity Questionnaire (IPAQ) was used to assess physical activity; scores > 3000 MET minutes per week would necessitate participants' exclusion due to non-sedentariness. A validated Nordic Diet Score (NDS) questionnaire (Bjørnara et al. 2015) was used similarly, and participants scoring > 5 points would also need to be excluded. Exclusion criteria also included smoking, pregnancy and chronic conditions that might affect safe participation.

Dietary Intervention

Participants were advised to adhere to Public Health England's portion size guidelines (PHE 2016) but to follow the AND without restricting energy. During initial assessments, participants were briefed about AND-compliant foods (Table 1), obtained individualised diet plans, and provided with materials (recipes, etc.) and food items (root vegetables, cruciferous vegetables, fish, rye bread and apples; enough for 2 weeks) to improve adherence and foster behaviour change (Michie et al. 2011). Participants were also instructed to complete a 3-day diet diary pre and post intervention (two assessments); data was inputted into software

(Nutritics, Dublin, Ireland) incorporating M^CCance and Widdowson's UK Composition of Food Database (2015) within its databank (Nutritics Ltd product version 1.7, Dublin Ireland), for dietary analysis. Kcals, Total Fat, Saturated Fat, Protein, Carbohydrates, Fibre and Omega 3 (Total *n*-3) were calculated, to measure dietary changes that might impact microvascular function (Calder et al. 2013). Follow-up consultations were conducted via telephone and email at weeks one and three to foster support, and a private social media group was created to engender social support similarly (Michie et al. 2011). Participants were advised to maintain activity as indicated by their pre-intervention IPAQ scores; no physical activity intervention was provided.

Table 1 Nordic Foods

| Vegetables | Fruit | Fish/Meat | Grains | Other |
|-------------|---------------|-----------|-------------------|--------------|
| Cabbages | Blueberries | Game | Wholegrain breads | Dill |
| Cauliflower | Blackcurrants | Poultry | Rye | Parsley |
| Broccoli | Redcurrants | Cod | Oats | Chive |
| Kale | Gooseberries | Salmon | Barley | Legumes |
| Onions | Apples | Herring | | Rapeseed oil |
| Swede | Pears | Haddock | | |
| Carrots | Plums | Mackerel | | |
| Beetroot | | Halibut | | |
| Turnip | | | | |
| Potatoes | | | | |
| Parsnips | | | | |
| Mushrooms | | | | |

Protocol

We used Laser Doppler Flowmetry (LDF) and Transcutaneous Oxygen Monitoring (TcP02) to assess microvascular function pre and post intervention, reflecting procedures described by

Wasilewski, Ubara and Klonizakis (2016). Laser Doppler Flowmetry was used to determine cutaneous microvascular responsiveness to local heating (Tew et al. 2011); Transcutaneous Oxygen Monitoring was used to assess tissue oxygen supply (Bajwa et al. 2014). To measure LDF and TcPO₂ pre and post-intervention, we required participants to attend the laboratory on two occasions, separated by a four-week intervention period, and instructed them to abstain from caffeine prior to attending, to eliminate acute vasoconstriction (Umemura et al. 2006). Stature (cm) body mass (kg), body fat % and BMI (kg · m²) were measured concurrently using a segmental body-composition analyser (InBody 720, Derwent Healthcare; UK) and compared at both time points.

LDF Procedure

Microvascular blood flow was measured as cutaneous red blood cell flux using a Laser Doppler Flowmeter (Periflux system 5000, Perimed 122 AB, Järfälla; Sweden) and a 7-point LDF probe (Probe 413, 123 Perimed AB), using procedures outline by Tew et al. (2010). Participants were acclimated to a temperature-controlled room (ambient temperature set to 22 - 24 °C) before collecting data. Participants' forearms were cleansed prior to attaching the LDF probe to the skin on the underside of the right arm, avoiding veins and hair, to circumvent abnormal readings. Local thermal hyperaemia was induced using a heating disk (Model 455, Perimed AB) connected to a heating unit (Model 5020, Perimed AB) and LDF signals were recorded using PeriSoft software (PSW 9.0). Baseline blood-flow data were recorded for five minutes with the local heating disc set to 30 °C. Temperature was then increased (1° C · 10 s⁻¹) to 42 °C to induce rapid local heating, which was then maintained for 30 minutes. After this, the probe temperature was increased to 44 °C for 10 minutes to achieve maximal vasodilation. Resting blood pressure (mmHg) and heart rate (bpm) were recorded at baseline and at every five minutes during data collection using a patient monitoring device (Dinamap Dash 2500, GE Healthcare; USA). Thermal hyperaemic data

were recorded during the test and expressed as cutaneous vascular conductance (CVC) at four regions (baseline, initial peak, plateau, and maximum regions) and presented as raw CVC and CVC normalised to maximum (%CVCmax: $[(\text{CVC} / \text{maximum CVC}) \times 100]$).

Transcutaneous Oxygen Measurement

The sub-maximal exercise test (Table 2) was performed after the LDF procedure using a cycle ergometer (824E, Monark AB; Sweden). Heart Rate (HR) (Sports Tester, Polar; Finland) and Ratings of Perceived Exertion (RPE; CR10 scale, Borg, 1998) were recorded at each minute and blood pressure (mmHg) was recorded one minute into every two-minute rest period using participants' contralateral arm, using the patient monitoring device (Dinamap Dash 2500, GE Healthcare; USA). Oxygen tension was measured using a calibrated TINA TCM400 tcpO2 device (Radiometer; DK) during the test. A temperature probe, set to 44.5 °C to achieve maximal skin vasodilatation, was attached to the skin of the participants' sub-scapular area using a fixation ring, which was attached to participants' back approximately 10 mm below the left scapula, avoiding bone, and using contact solution. The solution was allowed to heat, causing skin dilatation. Dilatation of the skin-blood capillaries increases blood flow, causing a diffusion of oxygen through the skin into the sensor, which then measures TcPO2. After this, TcPO2 measurements were temperature corrected to 37 °C by the TINA device. For the purposes of this study, TcPO2 was defined as the raw oxygen perfusion values obtained directly from the TINA recordings (Table 3).

Table 2 Submaximal Exercise Protocol

| Interval : Time (mins) | Resistance (kg) | Speed (RPM: revolutions per minute | Power output (Watts) |
|------------------------|-----------------|---------------------------------------|----------------------|
| Interval 1 : 5 mins | 1kg | 80 RPM | 80W |
| Rest : 2 mins | - | - | |
| Interval 2: 5 mins | 1.2kg | 80 RPM | 96W |
| Rest : 2 mins | - | - | |
| Interval 3: 5 mins | 1.4kg | 80 RPM | 112W |
| Rest : 2 mins | - | - | |
| Interval 4: 5 mins | 1.6kg | 80 RPM | 128W |

Table 3 TcPO2 Variables

| TcPO2 Quantity | Definition |
|-------------------|---|
| Baseline | The arithmetic mean of maximum TcPO2 at rest |
| TcPO2max | The greatest TcPO2 value each minute of exercise or rest. |
| Δ TcPO2max | The maximum change from baseline value e.g. TcPO2max – baseline. |
| Δ TcPO2 | Average sum of change in Transcutaneous oxygen tension from baseline. |

Statistical Analysis

Independent t-tests were performed on baseline physical characteristic and dietary analysis data. A two-by-two mixed design Analysis of Variance (ANOVA) compared the effects of the AND intervention on blood pressure (systolic and diastolic), body-mass, body-fat %, peak heart rate, RPE, Δ TcPO2, Δ TcPO2max, CVC, %CVCmax and diet data (NDS, Kcals, Total Fat, Saturated Fat, Carbohydrates, Protein, Fibre and Omega 3) in the older and younger participants using SPSS (SPSS Inc., Chicago Illinois, version 23 for Windows). The alpha level was set at $P = 0.05$. To accomplish normality or homogeneity of variance, Δ TcPO2, Body Fat %, Peak Heart Rate and dietary data (NDS, Kcals, Omega 3) were log transformed

207 prior to inferential analyses, after checking for and ensuring underlying assumptions.
208 Independent and dependent-samples t-tests followed up significant interactions. Data are
209 presented as mean \pm SD.

210

211

Results**Participants**

Thirteen young (18 - 35 years) and fifteen older (55 – 75 years) participants completed the study from the sixteen young and sixteen older participants originally recruited, equating to an 82 % and 94 % completion rate. Participants' characteristics are presented in Table 4.

Table 4 Participants' Characteristics Pre and Post Intervention

| | Group A (Young) | | Group B (Old) | |
|-------------------------------|----------------------------|----------------|--------------------------|----------------|
| | Visit 1 | Visit 2 | Visit 1 | Visit 2 |
| Gender | 5 male, 8 female | | 7 male, 8 female | |
| Age (years) | 28 (5) [†] | | 64 (6) [†] | |
| Resting BP (systolic) | 129 (10) [†] | 123 (9) | 150 (14) [†] | 148 (19) |
| Resting BP (diastolic) | 78 (15) | 72 (8) | 81(12) | 79 (16) |
| Stature (cm) | 171 (6.0) | | 168 (6.6) | |
| Body Mass (kg) | 69.1 (22.1) | 67.4 (22.1) | 81.6(16.8) | 80.6 (16.7) |
| BMI (kg·m²) | 24.3 (7.9) | 23.6 (7.9) | 30.5 (5.4) | 29.7 (5.4) |
| Body Fat (%) | 27.2 (8.3) | 25.2 (8.8)* | 36.5(8.9) | 35.3(8.5) |

[†]P = <0.05 between groups (at baseline),

*P = <0.05 between visits (within groups)

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Dietary Analysis

Baseline Kcals [young = 1615.2 (645.6), old = 2595.2 (567.3); *P* = 0.14], Total Fat [young = 61.2 g (22.6), old = 122.0 g (56.4); *P* = 0.03], Saturated Fat [young = 22.0 g (7.3), old = 38.5 g(13.8); *P* = 0.027] and Fibre [young = 15.4 g (4.9), old = 27.7 g (4.3); *P* = 0.001] were lower in the younger participants (Table 6). Post intervention, only Kcals [young = 1353.0 (274.3), old = 2042.7 (676.0); *P* = 0.29] and Total Fat [young = 45.5 g (11.6), old = 87.7 g (36.5); *P* = 0.022] differed between groups. Between visits, NDS [young = 2.5 (0.8) to (5.7 (1.4); *P* =

0.01, old = 2.3 (1.2) to 5.2 (0.8); $P = 0.02$] increased in both groups similarly. Fibre intake increased in the younger group (15.4 g (4.9) to 24.3 g (3.0); $P = 0.05$). No other dietary data reached statistical significance (Table 5).

Table 5 Raw Dietary Data Pre and Post Intervention

| | Young | | Old | |
|----------------------|------------------|----------------|------------------|----------------|
| | Pre-NND | Post- NND | Pre-NND | Post-NND |
| NND Score | 2.5 (0.8) | 5.7 (1.4) * | 2.3 (1.2) | 5.2 (0.8) * |
| Kcals | 1615.2 (645.6) † | 1353.0 (274.3) | 2595.2 (567.3) † | 2042.7 (676.0) |
| Total Fat | 61.2 (22.6) † | 45.5 (11.6) | 122.0 (56.4) † | 87.7 (36.5) |
| Saturated Fat | 22.0 (7.3) † | 13.9 (7.3) | 38.5 (13.8) † | 22.9 (10.8) |
| CHO | 194.8 (99.2) | 163.8 (54.8) | 237.3 (63.1) | 207.3 (102.5) |
| Protein | 80.7 (26.1) | 81.2 (13.3) | 101.3 (30.5) | 92.3 (28.9) |
| Fibre | 15.4 (4.9) † | 24.3 (3.0) * | 27.7 (4.3) † | 25.1 (6.3) |
| Omega 3 | 0.5 (0.5) | 0.5 (0.3) | 3.4 (5.0) | 2.4 (3.3) |

†P = <0.05 between groups (at baseline)

*P<0.05 between visits (within groups)

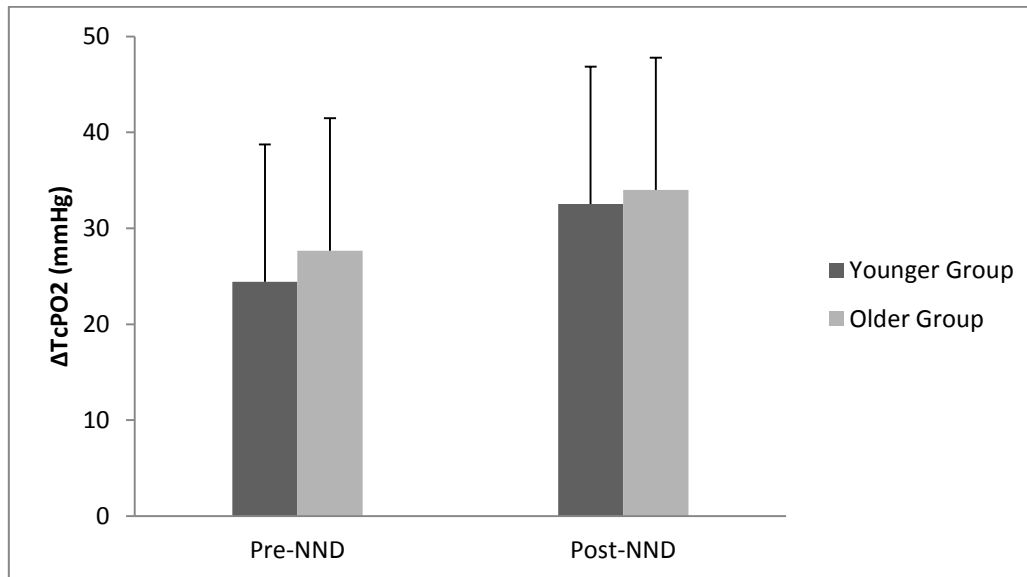
BMI, Body Mass, Body Fat and Blood Pressure

No differences in BMI or Body Mass were observed in either group at any time and no between-groups differences were noted for body fat % pre or post intervention. Only younger participants experienced reductions in body fat % between visits [27.2 (8.3) to 25.2 (8.8); $P = 0.028$] (Table 4). Baseline systolic blood pressure appeared to be lower in the younger participants [129 (10) vs. 150 (14); $P = 0.01$] however the AND had no effect on systolic blood pressure in either group (Table 4). Further, there were no changes in diastolic blood pressure in either group at any time (Table 4).

Oxygen Tension

There were no differences between the groups or changes to any of the TcPO₂ variables measured despite Δ TcPO₂ appearing to increase post intervention ($P = 0.26$) (Figure 1).

Figure 1 Δ TcPO₂ Pre and Post Intervention



Cutaneous Vascular Conductance

Baseline

Raw CVC

The younger group experienced no changes to raw CVC however the older group experienced an improvement during the 2nd assessment [0.35 (0.14) to 0.42 (0.16); $P = 0.02$] (Table 5). Baseline between-groups differences observed for Raw CVC were not present post-intervention (Table 6).

%CVC MAX

There were no differences between the groups or changes to %CVC MAX in either group at any time (Table 6).

Initial Peak

260 **Raw CVC**

261 Post-intervention, older participants exhibited lower Raw CVC [1.71 (0.53) vs 1.30 (0.30); P
262 = 0.01] at the initial peak compared to the young participants despite experiencing an
263 increase from baseline [1.17 (0.30) to 1.30 (0.30); P = 0.01] (Table 6). Pre-intervention
264 between-groups differences were not apparent post-intervention.

265 **%CVCmax**

266 No changes in % CVCmax were observed in either group at any time (Table 6).

267 **Plateau**

268 **Raw CVC**

269 Both groups experienced improvements in raw CVC at visit 2 (Table 5). No between-groups
270 differences were noted at the pre intervention stage for Raw CVC; however, between-groups
271 differences became apparent at the post-intervention period (young = 2.03 (0.62), old = 1.63
272 (0.39); P = 0.03) (Table 6).

273 **%CVCmax**

274 Improvements to %CVCmax were experienced in the younger participants only [78.8 (12.0)
275 to 85.0 (10.7); P = 0.03]. Similar to Raw CVC, no between-groups differences were noted at
276 the pre-intervention stage but were noted at the post-intervention period [young = 85.0 (10.7),
277 old = 77.7 (7.3); P = 0.03] (Table 6).

278

279 **Table 6** Cutaneous Vascular Conductance Pre and Post Intervention

| | Group A (younger group) | | Group B (older group) | |
|-----------------------------|--------------------------|--------------------------|---------------------------|-------------|
| | Raw CVC | % CVC MAX | Raw CVC | % CVC MAX |
| Baseline | | | | |
| Visit 1 (pre-intervention) | 0.33 (0.12) [†] | 12.7 (5.2) | 0.35 (0.14) [†] | 13.9 (4.3) |
| Visit 2 (post-intervention) | 0.39 (0.11) | 15.0 (8.2) | 0.42 (0.16) [*] | 11.0 (7.7) |
| Initial Peak | | | | |
| Visit 1 (pre-intervention) | 1.55 (0.47) | 72.7 (10.4) | 1.17 (0.30) | 63.0 (16.1) |
| Visit 2 (post-intervention) | 1.71 (0.53) [†] | 76.0 (13.6) | 1.30 (0.30) ^{†*} | 71.9 (10.4) |
| Plateau | | | | |
| Visit 1 (pre-intervention) | 1.67 (0.50) [†] | 78.8 (12.0) [†] | 1.49 (0.37) [†] | 71.7 (12.1) |
| Visit 2 (post-intervention) | 2.03 (0.62) [*] | 85.0 (10.7) [*] | 1.63 (0.39) [*] | 77.7 (7.3) |

280 [†]P = <0.05 between groups

281 ^{*}P<0.05 between visits (within groups)

282

283 ***Peak Heart Rate and RPE***

284 A reduction in peak heart rate was observed in the older group only [149.5 (7.9) to 146.1
285 (6.5); *P* = 0.01]. No differences between the groups or changes in RPE were noted.

286

288 Our study is the first to investigate the effects of a short-term, adapted Nordic diet
289 intervention on endothelial function and tissue oxygenation in adults at rest and during mild-
290 to-moderate exercise. These results highlight the short-term effects of the diet with respect to
291 a number of parameters which define CVD risk and day-to-day function in older and younger
292 individuals. Further, our findings support previous work elucidating the effects of sedentary
293 aging on cutaneous microvascular function: Similar to Tew et al. (2010), our older
294 participants demonstrated lower pre-intervention raw CVC during the initial peak and plateau
295 stages, suggesting age-related vasodilation impairment in response to local skin heating. The
296 mechanisms underpinning the weakened initial peak observed in older adults are not fully
297 understood however evidence suggests that local sensory nerve dysfunction, diminished
298 noradrenergic sympathetic nerve stimulation and reduced NO synthesis might attenuate the
299 rapid skin hyperaemic response in older individuals (James et al. 2006)—reduced
300 endothelial-mediated NO synthesis is thought to explain the diminished plateau. Age-related
301 microvascular impairment is associated with coronary events (James et al. 2006). Strategies
302 to improve microvascular function are clinically important therefore.

303 Tew et al. (2010) identified that maintaining aerobic fitness into advanced age might be one
304 such strategy, while findings elsewhere (Klonizakis et al. 2013, Klonizakis et al. 2016)
305 suggest that diet and exercise might also provide long-term benefits. Our data supports this
306 suggestion, and highlights encouraging benefits of a short-term dietary modification. Older
307 participants experienced improvements in raw CVC at baseline, initial peak and plateau
308 stages, suggesting that the AND led to improvements in axon-mediated vasodilation (during
309 the initial peak) and endothelial-mediated NO synthesis (during the plateau). Decreasing
310 axon-mediated vasodilation indicates microcirculatory dysfunction (Nouri et al, 2012) and
311 increased risk of cardiovascular events (Hadi et al. 2007). Our data therefore provide a

preliminary indication that diet might be a mechanism to attenuate this dysfunction in an aging population. Younger participants experienced improvements in a number of CVC parameters similarly, the magnitude of which appeared to be greater than the older individuals. While drawing comparisons with other studies is erroneous given the novelty and specificity of dietary-intervention research, our data reflects those observed elsewhere: Klonizakis et al. (2013, 2014) and Alkhatib and Klonizakis (2014) revealed that the MD, when coupled with exercise, led to greater improvements in endothelium-dependent vasodilation than an exercise-only condition in older sedentary individuals (55 ± 4 years). Collectively, data is beginning to suggest that wholefood; nutrient-dense diets might promote endothelial health by also increasing NO synthesis, given adequate consumption of nitrate-rich foods (Sobko et al. 2010). Indeed, age-related NO decline is also associated with CVD risk (James et al. 2006); augmenting endothelial-dependent NO production via dietary modification might mitigate such risk however.

The role of dietary fat in microvascular health is multifaceted: High-fat meals appear to promote endothelial dysfunction (Esposito et al. 2007) and a high-saturated-fat diet might impair endothelial vasodilation (Keogh et al. 2005). However, *n*-3 Polyunsaturated Fatty Acids (PUFA) might *improve* endothelial health (Calder et al. 2013). While our participants reduced saturated and total fat (trends only) intakes, neither group increased *n*-3 PUFA concomitantly. This was despite the AND encouraging fish consumption. Data indicates that *n*-3 PUFA might activate NO synthesis and reduce oxidative stress and inflammation (Zanetti et al. 2015). It is possible therefore that substituting a proportion of saturated and total fat in the diet for *n*-3 PUFAs might have led to superior changes to a number of microvascular parameters we assessed. Future research might need to ensure greater fish consumption for these to be realised however.

We adopted an incremental, sub-maximal exercise test such that tissue oxygenation could be assessed at rest and during activity. Reduced oxygen perfusion is associated with aging: Free-radical mediated endothelium-dependent NO degradation has been demonstrated in older adults, leading to arterial narrowing, increased blood pressure and cardiac complications (Gates et al. 2009). We observed no effects of the AND on any TcPO₂ parameters, contrasting data suggesting that short-term green tea consumption might lead to improved tissue perfusion (Wasilewski et al. 2016). This was surprising considering that the AND is a flavonoid-rich diet and that the flavonoids found in green tea explain its efficacy (Wasilewska et al. 2016). While not measured specifically, insufficient consumption of flavonoid-rich foods (e.g. berries, apples, and parsley) might explain our findings. Future studies should ensure sufficient consumption of these foods for improvements in TcPO₂ to be possible as part of an AND intervention.

We observed statistically-significant reductions in body-fat % in younger participants and improved peak heart rate in older participants, adding to existing data revealing health benefits of Nordic-type diets (Kanerva et al. 2014b). The reductions in body fat we observed (young = -2%) corresponded with a weight-loss trend (young = -2.5%). While the AND might have led to this weight loss, we cannot rule out that participating in a dietary intervention might have prompted some participants to lose weight intentionally, and that this weight loss might explain some of the improvements we noted. Nevertheless, owing to such changes being observed, and that Nordic eating appears to be associated with low obesity prevalence (Kanerva et al. 2013), and weight loss elsewhere (Poulsen et al. 2015), future research should investigate the potential of a Nordic diet for weight-management purposes specifically.

The efficacy of the AND to elicit improvements in microvascular function appeared to greatest in younger participants, contrasting our original hypothesis. This might be due to

361 younger participants experiencing larger changes to habitual eating patterns via compliance
362 to the AND: while pre-to-post intervention NDS differed for both groups, a higher mean
363 change was observed in younger participants. It is also possible that younger individuals
364 might be more responsive to the AND: lifestyle (diet, physical activity) *and* biological factors
365 (hormonal changes, etc.) are known to lead to endothelial dysfunction with age; older
366 participants might have experienced dampened responsiveness due to such age-related
367 factors. However, complex interventions (diet and physical activity) have been shown to lead
368 to important, long-lasting improvements in microvascular function in older individuals
369 (Klonizakis et al. 2014). Future research might need to account for older participants'
370 responsiveness to diet-only intervention; complex interventions might be needed for greater
371 changes to be realised.

372 Limitations of this research include the lack of objective measures to determine compliance
373 to the AND, and the absence of control groups. Compliance measurements could be explored
374 in future studies; the lack of control group might make inferences about the efficacy of the
375 AND to elicit functional change to the endothelium difficult without a comparator. However,
376 baseline measurements were used here for such comparisons, and it was felt that control
377 groups were unnecessary owing to previous data highlighting the efficacy of dietary
378 intervention to stimulate microvascular change in young and old groups (Wasilewski et al.
379 2016). The short study duration, which might also be considered a limitation by some, was
380 intentional, with the view to explore the minimum duration after which medium-term effects
381 can be identifiable across populations. Our results have provided such indications, and so
382 further studies with a longer-duration might now be explored. Finally, an additional limitation
383 here is the lack of objective exercise-behaviour monitoring employed outside of testing. Such
384 monitoring should be implemented in future investigations.

Conclusion

This study supports current evidence highlighting health benefits of regional diets. Our participants, who were sedentary, observed improvements in body composition and microvascular function by integrating Nordic foods into their diet for a 4-week period. There is now a need to investigate effects of Nordic-type diets over longer intervention periods, particularly among older individuals (55+ years), who appeared to be less responsive to the intervention. Age-related endothelial dysfunction might be a preliminary indicator of CVD events; strategies to attenuate age-related microvascular deterioration therefore require further investigation.

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