

**Physiological effects of a short-term, lifestyle intervention based on the Mediterranean diet - comparison between older and younger healthy, sedentary adults**

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**Full Title: Physiological effects of a short-term, lifestyle intervention based on the Mediterranean diet - comparison between older and younger healthy, sedentary adults.**

**Running Head - Short-term Mediterranean diet intervention**

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**Authorship** - MK designed the study. YL and MM collected the data. MK, YL and MM analysed and interpreted the data and wrote the manuscript. All authors read and approved the final manuscript.

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## **Abstract**

**Objective:** To determine whether short-term adherence to the Mediterranean Diet (MD) is associated with improved physiological function.

**Research Methods and Procedures:** This was a single-centre, cohort pilot study. Twenty-four healthy, sedentary younger (18-35 years) and older (55-75 years) adults took part. Participants were advised and supported to follow the Mediterranean diet for 4 weeks. We took baseline and post-intervention measurements of weight, height, waist and hip circumference, blood pressure, heart rate, as well as microvascular physiological assessments using laser Doppler Fluximetry (LDF) at rest as well as transcutaneous oxygen pressure (TcPO<sub>2</sub>) during a sub-maximal exercise assessment.

**Results:** We identified statistically-significant improvements in axon-mediated microvascular vasodilation (2.24 ( $\pm$ 0.56) to 3.14 ( $\pm$ 0.84),  $P = 0.03$ ) and endothelial-mediated NO synthesis (2.59 ( $\pm$ 0.67) to 3.32 ( $\pm$ 0.87),  $P = 0.022$ ) in the younger group. Despite the intervention not including an exercise element, the rate of perceived exertion was reduced in both groups ( $p < 0.001$ ), after following the MD for a month.

**Conclusions:** Improvements in physiological function were observed following a short-term dietary intervention based on the MD in a younger population. These were not matched in a senior group. Our findings suggest that different durations should be applied when designing dietary interventions in different age-groups, with expectations in physiological improvements differing.

**Keywords:** Mediterranean diet, LDF, Oxygen tension, cardiovascular disease, blood pressure.

## **Introduction**

Our health is determined by many different factors (e.g., genetical, environmental and lifestyle-related). Nutrition is a major, modifiable factor which contributes to our health and disease risk. The risk of developing cardiovascular disease (CVD) increases with age, but approximately 50% of the adult population and 30% of children are considered at high risk of developing CVD [1]. Additionally, events such as the menopause (which causes a decline in oestrogen, which has detrimental effects on the cardiovascular system), increases the risk of developing CVD in post-menopausal women [2]. CVD affects more than 7 million people in the UK, and the cost of managing cardiovascular diseases in the UK is more than £28 billion per year [3]. Thus, developing new methods of reducing the burden of CVD could have the potential to vastly increase the quality of life for many people and reduce healthcare costs.

Fortunately, certain risk factors – such as our diet - are modifiable: With saturated fat and salt intake being the two major dietary CVD risk factors [1], it is important to consume diets that use less such content. The Mediterranean diet (MD) is based on increased consumption of olive oil, fruit, vegetables and salad, fish, legumes, wholegrain foods, moderate consumption of wine and limited consumption of red and processed meat, confectionary and dairy products [4]. Therefore, the MD contains low levels of saturated fat, salt and free-sugar, and high unsaturated fat and fibre. This dietary composition is associated with

decreased build-up of fatty deposits in blood vessels [5], reducing the risk for CVD and associated health conditions (e.g., stroke, heart attack and atherosclerosis) [6, 7].

Endothelial dysfunction is a major risk factor for the development of CVD and hypertension [3], characterised by diminished nitric oxide supply and a lack of arterial vasodilation [8] and strongly affected by age [7]. Endothelial dysfunction is known to be reversible; a healthier diet such as MD can potentially reduce the dietary risk factors, enhancing endothelial function [9].

Research shows that long-term adherence to the MD reduces the development of cardiovascular disease. For example, recent studies suggest a 9% reduction in overall mortality [10], and a reduction of cardiovascular risk factors in the elderly population in those following the MD, either on its own [11] or in combination with exercise [12].

Although the benefits of MD adherence are undeniable, there are certain elements that need to be explored further: for example it is unknown what is the optimal duration of an MD regime and at which point do the cardiovascular benefits can be firstly observed. It is also unknown whether benefits are similar between different age groups. Such knowledge could help to provide a clearer structure for health practitioners to develop interventions at specific population- or age-groups, providing more effective interventions.

We therefore conducted a study, aiming to determine the short-term effects of the MD on the human physiology and compare the differences in physiological effects between senior and younger age groups.

We hypothesised that both groups will show improvements in microvascular, physiological function after the MD intervention, with effects being greater in the senior group.

## **Methods**

### **Ethical Approval**

This study was approved by the Sheffield Hallam University Ethics Review Committee. All participants provided written consent to take part in the study.

### **Study Design**

This was a single-centre, cohort study. Baseline measurements of physiological function were taken in an initial laboratory session. The subjects then followed the MD for one month, after which time, all measurements were repeated.

### **Participants**

Twelve participants were aged 18-35, and twelve were aged 55-75. All participants were healthy, sedentary and not taking any form of medication that may influence cardiovascular function. Volunteers were recruited via posters, emails to staff and students of the University of Sheffield and Sheffield Hallam University, and word of mouth in Sheffield,

United Kingdom. All participants were screened to ensure they did not already follow the Mediterranean diet (e.g., consuming olive oil, fruit, vegetables and salad, fish, legumes, poultry meat, wholegrain foods and wine), using MEDAS, a validated Mediterranean diet adherence questionnaire [13]. Individuals who scored more than 6 MEDAS were considered to already be consuming significant elements of the Mediterranean diet on a regular basis, therefore they were excluded. Participants' physical activity levels were assessed (in the form of Metabolic Equivalent of Task (MET)-minutes) via an International Physical Activity Questionnaire (IPAQ) [14] to ensure they met the sedentary requirements.

Exclusion criteria were an active lifestyle (i.e. not sedentary defined as undertaking more than 60 min of structured or planned physical activity per week), habitual adherence to the MD, any chronic disease that could affect vascular function (such as diabetes, CVD and hypertension), pregnancy and smoking.

### **Protocol**

Eligible participants were invited to the University of Sheffield Hallam Centre of Health and Wellbeing on two occasions. Participants had their height, weight, hip and waist circumference measured and recorded, and from this, BMI was calculated using the

formula:

$$\text{BMI} = \text{weight (kg)} / (\text{height (m)}^2).$$

MEDAS was used to measure the current adherence of participants to the MD, both prior to starting and at the end of the intervention. Participants also completed two 3-day estimated food diaries [15] one prior to beginning the diet, and one during the last week of the diet, to determine their adherence to the MD.

Participants were instructed to follow the Mediterranean Diet for 4 weeks, or until their second appointment, depending on their availability. They were given a food "starter" pack, providing them with staples such as brown rice, brown pasta, lentils, olive oil, nuts, chopped tomatoes and tomato puree. Participants were also given instructions on how to adapt their current diet to the MD. We considered making swaps (such as swapping white pasta and rice to wholegrain varieties), to be more achievable than a complete diet overhaul. We also provided participants with an information booklet, guidelines on what constitutes the MD and a recipe booklet. A Facebook group was set up to support participants and answer any questions. This group provided support by regularly adding recipes and tips, and encouraging participants to share any experiences or problems, to create a supportive community-feel to the group, allowing participants to support each other throughout the diet.

### **Transcutaneous oxygen pressure (TcPO<sub>2</sub>) and sub-maximal exercise tests**

A discontinuous sub-maximal test involving 4 rounds of exercise was completed to measure the participants' transcutaneous oxygen pressure (TcPO<sub>2</sub>). Measurements were taken by



attaching the participants to a probe. The probe was heated to 44.5°C, to non-invasively cause maximal dilation of the skin capillaries. The TcPO<sub>2</sub> values were measured and recorded by the sensor. Measurements were taken by the TINA TCM400 TcPO<sub>2</sub> device, (Radiometer, Copenhagen, Denmark). The electrode was placed on the participants' back, below the right scapula. TcPO<sub>2</sub> readings were taken directly from the raw oxygen tension values, measured by the device.

The participants were characterised as being either "healthy" or "diseased". In healthy participants, their initial TcPO<sub>2</sub> readings increased during exercise, whereas diseased participants showed a decline in initial TcPO<sub>2</sub> measurements during exercise [16].

Participants cycled for 20 minutes, with a 2-minute break after each 5 minutes of exercise.

The participants maintained a speed of 80RPM, and after each 5-minute period, an extra 0.2kg of resistance was added to the ergometer, beginning on 1kg of resistance.

The participants were encouraged to reach their maximum exertion limit before stopping, but

could stop before the end of the test if they needed to.

### **Self-perceived exertion**

During the sub-maximal exercise test, we measured the rate of perceived exertion (RPE) of participants at one-minute intervals whilst they were cycling [17]. The RPE scale counts from 6-20, with 6 representing no exertion and 20 representing maximal exertion [18]. The RPE

scale is approximately linked to heart rate (with 6 corresponding to 60 beats/minute, and 20 corresponding to 200 beats/minute [18]. Finally, RPE values were explained and participants were trained in giving them, prior to assessments.

### **Laser Doppler Flowmetry (LDF)**

Laser Doppler Flowmetry (LDF) combined with local heating of the skin was used to determine cutaneous microvascular responsiveness providing a simple approach for examining the microvascular and endothelial function [19]. The tests were conducted in a temperature-controlled room, with an ambient temperature of 22 –24°C, as previously described by our group [20]. A site on the upper right forearm was chosen avoiding visible vein, body hair and damaged or irritated skin. This site was sterilised, then the LDF probe was attached to the skin. Local thermal hyperaemia was induced using a heating disc (Model 455, Perimed AB) connected to a heating unit (Model 5020, Perimed AB) and LDF signals were recorded using PeriSoft for Windows 9.0 software (PSW 9.0). Throughout the LDF, the resting HR and BP (systolic and diastolic) were recorded from left arm at baseline and at 5-min intervals using a patient monitoring device (Dinamap Dash 2500, GE Healthcare; USA). Baseline skin blood flow data was recorded for five minutes with the local heating disc temperature set at 32°C. Then, the temperature of the probe was increased by 1°C every 10s until 42°C was reached to induce rapid local heating. This was maintained for 25 minutes. After this, the temperature was quickly increased to 44°C for the final 10 minutes to

obtain maximal vasodilatation, which completed the test. Regions of the response were then recorded (Table 1). The LDF values divided by the corresponding mean arterial pressure to give the cutaneous vascular conductance (CVC) in APU/mm Hg. The mean arterial pressure was calculated from systolic and diastolic blood pressure. The data presented as raw CVC and CVC normalised to maximum (%CVCmax = [(CVC / maximum CVC) x 100]). The resting HR and BP of participants were calculated from the average of measured HR and BP throughout the LDF.

### **Statistical analysis**

As this was a pilot study, no formal sample size was calculated and the “sample size of 12 per group” was followed [21].

Paired t-tests were undertaken to compare the effects of the MD on BMI, waist and hip circumference, TcPO<sub>2</sub>, RPE, CVC, %CVC Max and MD score between the younger and senior groups at the same time-point. The baseline and post-intervention data were compared, also comparing the differences between the senior and younger group. The data recorded during the initial laboratory assessment, prior to the intervention, was used as the baseline measurements. Statistical analysis was performed using SPSS. Statistical significance for the test was set at  $P \leq 0.05$ . Values were presented as mean ( $\pm$  SD).

## **Results**

### **Participant Characteristics**

Twelve participants aged 18-35 were eligible to take part in the study, making up the younger group. All participants completed all study assessments (Table 2). An equal

number of older (e.g., 55-75 years of age) participants have completed all study assessments (Table 2).

### **Anthropometric measures**

No significant differences in BMI, waist circumference, hip circumference, heart rate or resting systolic or diastolic blood pressure from pre-intervention to post-intervention measurements in either the younger or the senior group (Table 2). There was no significant difference between groups in hip circumference, heart rate or diastolic blood pressure. However, there was a significant difference between groups in BMI, waist to hip ratio, waist circumference, and systolic blood pressure, with significantly lower values in the younger group for each measurement.

### **Blood pressure, BMI and weight**

There was no significant difference between BMI post-intervention, compared to baseline, in either the younger group or the senior group. There was a significant difference between the BMI of the younger and senior groups at baseline ( $p < 0.05$ ), and after the intervention ( $p < 0.05$ ).

There was also no significant difference between the younger group's systolic or diastolic blood pressure. Likewise, the senior group showed no statistically significant difference between the baseline and end of intervention in systolic or diastolic blood pressure.

There was a statistically-significant difference between the pre-intervention values for systolic blood pressure between groups ( $p < 0.001$ ). However, there was no significant difference between the baseline values for diastolic blood pressure between the younger and senior groups.

### **Side effects**

No participants withdrew from the study and no side effects were reported during the study period.

### **Compliance**

Mean MD score increased significantly in both the younger group ( $p < 0.001$ ) and the senior group ( $p < 0.001$ ) (Table 3). There was no significant difference in MD score between groups prior to the intervention, but there was a significant difference between the senior and younger groups MD scores at the end of the intervention, with the senior group reporting a higher MD score at the end of the intervention ( $p < 0.05$ ).

Compliance was also calculated through 3-day estimated diet records completed by the participants. The main findings from the records are summarised below (Table 3), in regards to energy, carbohydrate, fibre, protein, total fat, saturated fat, monounsaturated fat, polyunsaturated fat, fruit and vegetables, olive oil. Compliance to the MD was excellent, with statistically significant increases in intake of fruit and vegetables, fibre and olive oil, and

significant decreases in saturated fat. There was however, no significant change in monounsaturated fat or polyunsaturated fat intake.

### **Cutaneous vascular conductance (CVC)**

#### ***Baseline***

##### *Raw CVC*

There was no statistical difference between the groups for Raw CVC baseline records at any visit. Raw CVC baseline readings within the groups improved but there were not significant in either of the two groups (Table 4).

##### *%CVC Max*

There was no statistical difference between the groups or changes to %CVC Max baseline records in either group at any time. The post-intervention %CVC Max values were diminished for younger groups, but elevated for senior groups (Table 4).

#### ***Initial Peak***

##### *Raw CVC*

There was statistically-significant improvement for Raw CVC at initial peak within our younger group (2.24 ( $\pm 0.56$ ) to 3.14 ( $\pm 0.84$ ),  $P = 0.03$ ). The senior group's Raw CVC values were increased but not statistically significant. Additionally, no significant difference between groups was noticed at the beginning of the intervention for Raw CVC; nevertheless, there was a statistically significant difference between groups at the end of the intervention

with the senior group indicating a lower value (3.14 ( $\pm$ 0.84) vs 2.52 ( $\pm$ 0.59),  $P=0.046$ ) (Table 4).

#### *%CVC Max*

No statistical differences between the groups or changes to %CVC Max at the initial peak readings in either group at any time was observed (Table 4).

#### **Plateau**

##### *Raw CVC*

Similarly to Raw CVC at initial peak, only the younger group had a statistically significant improvement for Raw CVC at plateau (e.g., 2.59 ( $\pm$ 0.67) to 3.32 ( $\pm$ 0.87),  $P=0.02$ ). The increase in the senior group values did not reach statistical significance, and neither were the differences between groups at both visits (Table 4).

##### *%CVC Max*

There were no statistical differences between the groups or changes to %CVC Max plateau records in either group, at any time (Table 4).

#### **Oxygen Tension**

##### *Baseline*

There were no statistically-significant differences between the TcPO<sub>2</sub> values for the younger or senior group between the pre- and post-intervention measurements, either within- or between-groups.

### *$\Delta TcPo_2$*

There was also no significant difference in  $\Delta TcPo_2$  for either group, between the two study visits. There was no significant difference between groups at either the pre- or post-intervention measurements.

### *TcPO<sub>2</sub>max*

There was no significant difference between the TcPO<sub>2</sub>max pre- and post-intervention in the young group or the senior group. There was also no significant difference between the groups, either pre- or post-intervention.

### *$\Delta TcPo_2$ max*

There was no significant difference in  $\Delta TcPo_2$ max in either group between visits, or between groups at either visit.

### *TcPO<sub>2</sub> profiles*

Following the categorisation by Ouedraogo and colleagues [16], we characterised participants as either "healthy" or "diseased", according to their TcPO<sub>2</sub> profiles. All participants in both groups were found to be "healthy" both before and after the intervention.



## **Rate of Perceived Exertion (RPE) and Heart Rate**

There was a statistically-significant reduction between the RPE from pre- to post-intervention in both the younger and senior groups (Table 5). No differences were observed between groups at baseline, however, a statistically-significant difference was noted post-intervention (Table 5).

No statistically-significant differences were observed either between- or within-groups at any stage in regards to heart rate (Table 5).

However, peak heart rate during exercise was significantly higher in the younger group in comparison to the senior group at all times (e.g., visit 1 (119 vs 96,  $p < 0.05$ ) and visit 2 (117 vs 97,  $p < 0.05$ ) (Table 5).

## **Discussion**

Our study participants reported enjoying their month following the MD anecdotally feeling healthier in themselves, which is in line with previous research by our group [22]. The 3-2day food diary reported during the intervention suggested excellent adherence to the diet in terms of saturated fat, fibre, fruit and vegetables, and olive oil intake. This suggests that the MD is an achievable diet to maintain in the long-term, which is an important pre-requisite of any successful public health initiative.

### *Blood pressure, in-exercise heart rate and RPE*

There was no statistically significant change in systolic or diastolic blood pressure in either the younger or the senior group. However, there was a reduction in diastolic blood pressure in both groups, although significance was not reached. These findings are consistent with other studies, which found that MD [23] or MD-inspired diets [24] caused a reduction in blood pressure. These results suggest that the MD intervention could be beneficial in reducing hypertension risk, and in turn, risk of developing CVD. However, some participants anecdotally reported "white coat syndrome" when their blood pressure was measured, which may suggest that any improvements in blood pressure caused by the MD were outweighed by increases in blood pressure due to anxiety. This might have been greater in our older female participants, as higher age and the female gender are two known "white coat syndrome" parameters [25].

Our study also notes a significant reduction in RPE, between visits, in both groups, although no such change was observed with exercising heart rate. The significance of this finding is unknown, although Kakarot and Müller suggest that RPE (a psychological estimation of exertion) is a better estimation of exertion than heart rate (a physiological estimation of exertion), as RPE alters in strong positive correlation with current exertion, whereas heart rate continues to increase after exertion has decreased [26]. Considering that we didn't

offer exercise sessions as part of our intervention, it could be that the MD triggered additional lifestyle changes, which affected perceived exertion as well.

### *Cutaneous microvascular function*

Our findings were consistent with previous studies interpreting the effects of sedentary aging on cutaneous microvascular function. Similar to Tew et al. [27], lower raw CVC of pre- and post- intervention during the initial peak and plateau stages were observed on the older participants, implying age-related vasodilation impairment in response to local skin heating. This is important because age-related reduction in microvascular function is associated with increased risk of CVD [28].

In the present study, statistically-significant improvements were observed in Raw CVC at initial peak and plateau stages for younger participants, suggesting that following the MD leads to improvements in axon-mediated vasodilation during the initial peak and endothelial-mediated NO synthesis during the plateau. Decreasing axon-mediated vasodilation suggests microcirculatory dysfunction [29] and thus, increased risk of cardiovascular events [9]. Because the plateau stage is largely mediated by NO, this reveals that the NO levels in the younger group was statistically-significant increased. It is well established that maintaining NO at adequate levels can reduce risk of CVD as reduced bio-availability of NO leads to a loss of cardio-protective actions and may even elevate the

progression of CVD [30]. This is consistent with previous literature in which an increase in endothelial function was observed following the MD [2, 12, 31]. This suggests that diet is a potential mechanism to improve endothelial function in the younger population. However, although endothelial function and axon-mediated vasodilation of older participants slightly increased after the MD, they were not statistically-significant, which is consistent with other studies [32].

In overall, although higher mean changes were found in senior group, statistically-significant improvements were only observed in the younger participants. This suggests that the younger population may be more responsive to the MD, and older individuals might have dampened responsiveness due to other age-related factors, (e.g., hormonal changes, lifestyle etc.) during a short-term implementation period [28, 33]. Future research may concentrate on comparing implementation periods for the senior population in conjunction with other lifestyle initiatives (e.g., smoking cessation, exercise) and assess the optimal duration after which significant improvements are detected.

#### *Transcutaneous oxygen pressure (TcPO<sub>2</sub>)*

It is well established that older adults are more susceptible to a lower oxygen perfusion than younger people, as blood vessels narrow, and arteries reduce elasticity due to increased risk of plaques causing blockages as we age [5]. This reduction in blood vessel efficacy may eventually be enough to cause reduced blood flow, causing hypertension. During

submaximal exercise, e.g. climbing stairs, carrying heavy shopping, etc. oxygen demand is high. Therefore, if there is a problem with TcPO<sub>2</sub>, it will become apparent during submaximal exercise. No measure of TcPO<sub>2</sub> changed significantly between pre-intervention and post-intervention measurements in either the younger or the senior group. This suggests that the MD as a short-term intervention may not be beneficial for improving oxygen tension, which means that cardiovascular risk remained the same before and after the MD in the study. However, in the long-term, dietary change (alongside weight loss) has been shown to be successful in improving microvascular function in overweight adults, similar to ours [34]. This research however, found that the benefits of the MD are greater for individuals who are less healthy at baseline, and also that exercise is also beneficial to improving microvascular function [35]. This suggests that a 4-week MD intervention may not be long enough to improve oxygen perfusion, and ultimately reducing an individual's risk of CVD, as CVD develops over a life-time.

#### *Experimental considerations and limitations*

All study participants were in general good health: this may mean that findings may be different in clinical or unhealthy populations. It is however, a positive first step for further research aiming at the general population. We also chose to use a 3-day diet diary instead of the "gold standard" -e.g., the 7-day weight food diary. Our choice however, was made in order to reduce participants' burden and decrease the likelihood of drop-outs.

We acknowledge that additional anthropometric measurements/assessments could have been completed, however, as the focus of the study was on vascular function and these tests tend to be lengthy we decided against adding further tests, to limit patient burden. Finally, although participants were asked to avoid caffeine intake for 3h before the assessments, it was difficult to factually control whether this was actually observed or not..

### **Conclusions**

In overall, we found evidence that a short-term intervention based on MD offered physiological benefits in a younger population. In contrast, no significant improvements were observed in a senior, healthy population, which was against to our original hypothesis. Therefore, and having in mind that an 8-week MD-based intervention improves microvascular function in senior healthy groups [12], further work needs to be undertaken to establish the optimum point at which physiological benefits can be observed by consuming the MD, as well as whether this can be achieved on its own or in combination of other intervention elements (e.g., exercise). Considering the known long-term benefits of the MD, it would be interesting to identify a minimum duration at which adherence to the MD results in benefits to cardiovascular physiology. In the long-term, the MD as a strategy to reduce the burden of CVD could have huge social and economic benefits to the NHS and social care system, as well as for individuals. Therefore, further research into a shorter-term MD intervention could be beneficial – as our findings suggest. There is a crucial need for

research into CVD reduction strategies, and the need for these findings to be promoted in clear public health messages.

### **Declarations of Interest**

None.

### **Ethical standards**

All experiments comply with the current laws of the country in which they were performed.

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**Table 1**

Laser Doppler Flowmetry regions of recorded responses.

<b>Response</b>	<b>Time</b>
Baseline	The arithmetic mean of the last 2 min of the first 5 min.
Initial peak	The arithmetic mean of the highest consecutive 30-s period within the distinct initial hyperaemic response.
Plateau	The arithmetic mean of the last 2 min of heating at 42 °C
Maximum	The arithmetic mean of the last 2 min of heating at 44 °C.

**Table 2**

Participant characteristics for the younger and older groups.

	<b>Group A (younger group)</b>		<b>Group B (older group)</b>	
	<b>Visit 1</b>	<b>Visit 2</b>	<b>Visit 1</b>	<b>Visit 2</b>
<b>Gender</b>	2 males, 10 females		4 males, 8 females	
<b>Age (years)</b>	25 ( $\pm 2.57$ )		59 ( $\pm 3.97$ ) <sup>#</sup>	
<b>Height (cm)</b>	1.70 ( $\pm 0.1$ )		1.66 ( $\pm 0.1$ )	
<b>Weight (kg)</b>	66.9 ( $\pm 12.8$ )	67.1 ( $\pm 13.0$ )	75.3 ( $\pm 14.9$ )	74.6 ( $\pm 15.2$ )
<b>BMI (kg/m<sup>2</sup>)</b>	23.1 ( $\pm 3.2$ )	23.2 ( $\pm 3.2$ )	27.3 ( $\pm 4.5$ ) <sup>#</sup>	27.1 ( $\pm 4.7$ ) <sup>#</sup>
<b>Waist circumference (cm)</b>	73.8 ( $\pm 10.4$ )	73.6 ( $\pm 9.7$ )	90.0 ( $\pm 12.3$ ) <sup>#</sup>	88.8 ( $\pm 13.6$ ) <sup>#</sup>
<b>Hip circumference (cm)</b>	97.5 ( $\pm 7.8$ )	99.1 ( $\pm 7.2$ )	103.8 ( $\pm 8.3$ )	102.4 ( $\pm 9.2$ )
<b>Resting HR</b>	69 ( $\pm 9.6$ )	72 ( $\pm 13.2$ )	61 ( $\pm 7.6$ )	62 ( $\pm 8.1$ ) <sup>#</sup>
<b>Waist to hip ratio</b>	0.76 ( $\pm 0.04$ )	0.74 ( $\pm 0.05$ )	0.87( $\pm 0.04$ ) <sup>#</sup>	0.87( $\pm 0.05$ ) <sup>#</sup>
<b>Resting BP (mmHg)</b>	108 ( $\pm 8.5$ )	109 ( $\pm 6.7$ )	126 ( $\pm 10.3$ ) <sup>#</sup>	126 ( $\pm 13.6$ ) <sup>#</sup>
<b>Resting diastolic BP (mmHg)</b>	68 ( $\pm 6.8$ )	68 ( $\pm 4.9$ )	76 ( $\pm 10.4$ ) <sup>#</sup>	77 ( $\pm 10.7$ ) <sup>#</sup>

<sup>#</sup> $P \leq 0.05$  between groups (at the same time-point).

HR: heart rate; BP: blood pressure.

**Table 3.**

Compliance to MD calculated from the 3-day diet diaries.

	Visit 1	Visit 2	Mean change	Visit 1	Visit 2	Mean change
<b>MD score</b>	4 ( $\pm 1.5$ ) <sup>*</sup>	11 ( $\pm 1.1$ )	+7	4 ( $\pm 0.9$ ) <sup>*</sup>	12 ( $\pm 1.0$ ) <sup>#</sup>	+8
<b>Energy/nutrient</b>						
<b>Total energy (kcal/day)</b>	2088 ( $\pm 590$ )	1868 ( $\pm 517$ )	-220	1821 ( $\pm 500$ )	1533 ( $\pm 342$ )	-288
<b>CHO (g/day)</b>	221 ( $\pm 54$ )	199 ( $\pm 53$ )	-22	190 ( $\pm 70$ )	145 ( $\pm 41$ ) <sup>#*</sup>	-45
<b>Fibre (g/day)</b>	20.8 ( $\pm 5.8$ )	26.8 ( $\pm 6.7$ ) <sup>*</sup>	+6.0	18.5 ( $\pm 6.5$ )	22.7 ( $\pm 6.8$ ) <sup>*</sup>	+4.2
<b>Protein (g/day)</b>	83.0 ( $\pm 36.5$ )	82.3 ( $\pm 31.3$ )	-0.7	63.8 ( $\pm 21.5$ )	68.8 ( $\pm 16.4$ )	+5.0
<b>Fat (g/day)</b>	88 ( $\pm 33$ )	74 ( $\pm 29$ ) <sup>*</sup>	-14	79 ( $\pm 29$ )	66 ( $\pm 19$ )	-13
<b>Saturated fat (g/day)</b>	30.7 ( $\pm 13.1$ )	20.0 ( $\pm 11.7$ ) <sup>*</sup>	-11	31.3 ( $\pm 14.7$ )	18.1 ( $\pm 5.7$ ) <sup>*</sup>	-13.2
<b>MUFA (g/day)</b>	30.3 ( $\pm 12.4$ )	32.0 ( $\pm 11.5$ )	+1.7	28.6 ( $\pm 12.0$ )	29.3 ( $\pm 7.9$ )	+0.7
<b>PUFA (g/day)</b>	13.4 ( $\pm 5.4$ )	14.9 ( $\pm 6.4$ )	+1.5	12.1 ( $\pm 4.4$ )	11.9 ( $\pm 6.4$ )	-0.2
<b>Food items</b>						
<b>Olive oil (serving/day)<sup>§</sup></b>	0.2 ( $\pm 0.3$ )	1.8 ( $\pm 1.2$ ) <sup>*</sup>	+1.6	0.1 ( $\pm 0.5$ )	1.7 ( $\pm 1.2$ ) <sup>*</sup>	+1.6
<b>Fruit and vegetable (serving/day)<sup>§§</sup></b>	3.6 ( $\pm 1.0$ )	5.5 ( $\pm 1.7$ ) <sup>*</sup>	+1.9	4.7 ( $\pm 4.3$ )	7.7 ( $\pm 3.5$ ) <sup>*</sup>	+3.0

\*  $P \leq 0.05$  between visits (within groups). #  $P \leq 0.05$  between groups (at the same time-point).<sup>§</sup>Serving based on 13g estimate (1 tablespoon of olive oil). <sup>§§</sup>Serving based on 80g estimate (1 portion of 5-a-day).

CHO: carbohydrates; MUFA: monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid.

**Table 4**

Cutaneous vascular conductance data for the younger and senior groups.

	Group A (younger group)		Group B (senior group)	
	Raw CVC	%CVC Max	Raw CVC	%CVC Max
<b>Baseline</b>				
Visit 1 (pre)	0.19 (±0.09)	6.64 (±3.65)	0.23 (±0.12)	8.06 (±4.84)
Visit 2 (post)	0.24 (±0.13)	6.03 (±4.25)	0.32 (±0.22)	10.4 (±11.4)
Change (post-pre)	0.05 (±0.15)	-0.62 (±5.66)	0.08 (±0.25)	2.32 (±11.4)
<b>Initial peak</b>				
Visit 1 (pre)	2.24 (±0.56)	77.9 (±20.4)	2.09 (±0.57)	72.1 (±15.8)
Visit 2 (post)	3.14 (±0.84)*	74.6 (±16.0)	2.52 (±0.59)#	74.3 (±16.5)
Change (post-pre)	0.90 (±0.83)	-3.28 (±11.3)	0.43 (±0.86)	2.18 (±24.4)
<b>Plateau</b>				
Visit 1 (pre)	2.59 (±0.67)	89.5 (±21.1)	2.45 (±0.66)	84.8 (±18.3)
Visit 2 (post)	3.32 (±0.87)*	78.4 (±11.7)	2.98 (±1.04)	84.2 (±10.7)
Change (post-pre)	0.73 (±0.95)	-11.1 (±30.0)	0.53 (±1.11)	-0.60 (±20.0)

\*  $P \leq 0.05$  between visits (within groups). #  $P \leq 0.05$  between groups (at the same time-point).

CVC: cutaneous vascular conductance

**Table 5.**

Sub-maximal exercise data comparing physiological function between visits and between groups.

	Younger group	P value (between visits)	Senior group	P value (between visits)	P value (between groups)
<b>Baseline TcPO<sub>2</sub> (mm Hg)</b>					
<b>Visit 1</b>	73.1 (20.1)	0.788	66.4 (14.7)	0.965	0.363
<b>Visit 2</b>	70.8 (12.4)		66.7 (7.0)		0.320
<b>TcPO<sub>2</sub>max (mm Hg)</b>					
<b>Visit 1</b>	87.6 (27.0)	0.767	77.8 (13.5)	0.744	0.271
<b>Visit 2</b>	84.6 (12.1)		79.4 (7.5)		0.220
<b>ΔTcPo<sub>2</sub> (mm Hg)</b>					
<b>Visit 1</b>	7.7 (3.8)	0.427	6.2 (4.5)	0.801	0.381
<b>Visit 2</b>	9.1 (4.6)		7.1 (9.6)		0.518
<b>ΔTcPo<sub>2</sub>max (mm Hg)</b>					
<b>Visit 1</b>	14.5 (9.1)	0.736	11.3 (4.3)	0.569	0.287
<b>Visit2</b>	13.8 (5.3)		12.8 (7.9)		0.699
<b>Mean exercise HR (beats/min)</b>					
<b>Visit 1</b>	119 (16)	0.766	96 (20)	0.674	0.013 <sup>†</sup>
<b>Visit 2</b>	117 (10)		97 (20)		0.047 <sup>†</sup>
<b>RPE (exertion)</b>					
<b>Visit 1</b>	12.3 (1.9)	<0.001 <sup>**</sup>	11.8 (1.6)	<0.001 <sup>**</sup>	0.181
<b>Visit 2</b>	11.8 (1.6)		10.6 (1.6)		0.018 <sup>†</sup>
<b>TPcO<sub>2</sub> profile characterisation</b>					
<b>Visit 1</b>	12 healthy	1.0	12 healthy	1.0	1.0
<b>Visit 2</b>	12 healthy	1.0	12 healthy	1.0	1.0

\*p<0.05 between visits (within groups), \*\*p<0.001 between visits (within groups), † p<0.05 between groups (at the same visit).

TPcO<sub>2</sub>: Transcutaneous oxygen pressure.