

Short Communication

The impact of age on the postprandial vascular response to a fish oil-enriched meal

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Although chronic fish oil intervention had been shown to have a positive impact on vascular reactivity, very little is known about their acute effects during the postprandial phase. Our aim was to examine the impact of a fish oil-enriched test meal on postprandial vascular reactivity in healthy younger (<50 years) v. older (≥ 50 years) men. Vascular reactivity was measured at baseline (0 h), 2 and 4 h after the meal by laser Doppler iontophoresis and blood samples taken at 0 and 4 h for the measurement of plasma lipids, total nitrite, glucose and insulin. Acetylcholine (ACh, endothelial-dependent vasodilator) and sodium nitroprusside (SNP, endothelial-independent vasodilator)-induced reactivities were greater at 4 h than at baseline or 2 h in the younger men ($P < 0.04$). These changes were not observed in the older men. Comparison of the male groups revealed significantly greater responses to ACh ($P = 0.006$) and SNP ($P = 0.05$) at 4 h in the younger compared with the older males. Postprandial NEFA concentrations were also greater at 4 h in the younger compared with the older men ($P = 0.005$), with no differences observed for any of the other analytes. Multiple regression analysis revealed age to be the most significant predictor of both ACh and SNP induced reactivity 4 h after the meal. In conclusion, the ingestion of a meal enriched in fish oil fatty acids was shown to improve postprandial vascular reactivity at 4 h in our younger men, with little benefit evident in our older men.

Age: Vascular reactivity: Fish oil fatty acids

Ageing, a major risk factor for CVD, is associated with a progressive decline in endothelial function characterised by an increase in smooth muscle tone⁽¹⁾ and decrease in fasting vascular reactivity^(2–4). These age-related changes are thought to be attributable to increased oxidative stress, lower antioxidant capacity⁽⁵⁾ and decreased endothelial NO synthase activity leading to a reduced bioavailability of NO in the vascular endothelium^(6,7). Interestingly, very little is known about the effects of the ageing process on vascular reactivity during the postprandial phase, although in a recent study, there was a trend for the increase in forearm blood flow in response to a high-fat meal to be blunted in older (50–68 years) compared with younger (20–42 years) men, even though measures of fasting vascular function were similar between the two age groups⁽⁸⁾.

Evidence is now emerging in the literature that the mechanisms involved in the control of vascular tone are influenced by dietary factors, with total dietary fat intake and fatty acid composition emerging as potentially important modulators. In particular, there is a limited but convincing body of evidence to indicate positive benefits of diets rich in the long chain *n*-3 PUFA, EPA and DHA on endothelial function

and vascular reactivity, when assessed after an overnight fast^(9–12). However, the acute impact of fish oil fatty acids on postprandial vascular reactivity is poorly understood. Three studies have examined the impact of EPA and DHA on postprandial vascular reactivity^(13–15), with inconsistent findings. In the study of Vogel *et al.*⁽¹³⁾, the consumption of a meal containing canned red salmon was shown to have little impact on flow-mediated dilatation in healthy subjects. In type 2 diabetics, an increase in vascular reactivity was observed with the addition of fish oil to a MUFA-rich meal, but this association was only observed in subjects with a fasting TAG concentration greater than or equal to 1.69 mmol/l⁽¹⁴⁾. We have recently observed a significant increase in endothelium-independent vasodilatation 4 h following the addition of fish oil fatty acids to a SFA-rich meal in healthy men⁽¹⁵⁾.

The purpose of the present study was to examine the impact of age on fasting and postprandial vascular reactivity and to determine whether the consumption of a fish oil-enriched test meal containing 5.4 g EPA and DHA (equivalent to two portions of oily fish) could result in an improved postprandial vascular tone relative to baseline in both younger (<50 years)

Abbreviations: ACh, acetylcholine; AUC, area under the curve; SNP, sodium nitroprusside.

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and older (≥ 50 years) men. Underlying mechanisms were investigated by determining the concentration of potential mediators of vascular reactivity in the circulation.

Subjects and methods

Subjects

Twelve younger (< 50 years, mean age of 30 (SD 10) years and a BMI of 23.2 (SD 2.6) kg/m^2) and twelve older healthy men (≥ 50 years, mean age of 63 (SD 5) years and a BMI of 27.5 (SD 3.2) kg/m^2) participated in a single meal postprandial study. Subjects were excluded if they had any metabolic disorders (e.g. diabetes or any other endocrine or liver diseases), consumed > 1 portion of oily fish per week or taking fish oil supplements providing > 1 g EPA and DHA per day, consumed high-dose antioxidant vitamin supplements (> 800 μg vitamin A, 60 mg vitamin C, 10 mg vitamin E or 400 μg β -carotene), were heavy drinkers (> 30 units of alcohol per week), or were taking medication that could affect lipoprotein metabolism or blood pressure. The subjects were asked to abstain from alcohol and organised exercise regimens for 24 h before each postprandial investigation. A low-fat evening meal (< 10 g fat) was consumed on the evening before each study day.

The test meal was given to the volunteers in the form of a warm chocolate drink containing the test oils and toasted white bread with strawberry jam. This meal provided 3.36 MJ of energy in the form of 114.1 g carbohydrate, 19.7 g of protein and 42.8 g fat (42% of dietary energy). The 40 g of test oils added to the drink consisted of 31 g of an 80:20 mixture of palm olein and soyabean oil (Aarhus United UK Limited, Hull, UK) and 9 g anchovy fish oil (Ocean Nutrition Canada Limited, Dartmouth, NS, Canada), providing 12.4 g SFA, 13.6 g MUFA and 12.8 g PUFA. The amount of DHA plus EPA (ratio 1.5:1) in the test oil was 5.4 g, equivalent to levels found in two portions of oily fish.

Following a 12 h overnight fast, the subjects attended the clinical investigation unit and a measure of forearm microvascular function was performed using laser Doppler imaging with iontophoresis. A baseline (0 h) blood sample was taken before the test meal was given, which the participants were requested to consume within 15 min. Microvascular function was assessed again 2 and 4 h after the meal before a second blood sample was taken at 4 h. No food was allowed during the postprandial test period and decaffeinated sugar-free drinks provided *ad libitum*.

The study was conducted according to the guidelines laid down in the declaration of Helsinki, and all procedures involving human subjects were approved by the University of Reading Research and Ethics Committee. Written informed consent was obtained from the subjects before the study began.

Assessment of vascular reactivity

Peripheral microvascular function was assessed using a validated technique that quantifies the vasodilatory responses to 1% acetylcholine (ACh, endothelium-dependent vasodilation) and 1% sodium nitroprusside (SNP, endothelial-independent vasodilation), delivered transdermally using iontophoresis.

Measures of vascular reactivity in the peripheral microcirculation have been shown to correlate with the coronary dysfunction^(16,17) and so provide a robust surrogate marker of vascular function in larger vascular beds. This non-invasive *in vivo* method has been described in detail elsewhere^(18,19). Briefly, subjects were taken to a temperature-controlled room (22–24°C) to rest for 30 min to ensure stable baseline conditions. For the vascular reactivity measurement, participants lay in a semi-recumbent position with their right arm supported on an armrest. A temperature probe and iontophoresis chambers were attached to the volar aspect of the forearm with ACh and SNP introduced into the anodal and cathodal chambers, respectively. Following a basal measurement of skin perfusion, an incremental constant current was delivered progressively in 5 μA steps (5, 10, 15 and 20 μA) to yield a total charge (current \times time) of 8000 C during the measurement. A series of fifteen scans was performed as the current increased from 0 to 20 μA and skin perfusion was measured using a laser Doppler imager (Moor Instruments Limited, Axminster, UK). For each scan, median flux values were determined within each of the iontophoresis chambers and plotted against the cumulative charge (0–8000 C).

Plasma separation and analytical procedures

Blood samples were collected into potassium EDTA tubes and plasma separated by centrifugation at 1700 g for 10 min in a bench top centrifuge at 4°C. Plasma for the analysis of lipids, glucose, insulin and nitrite was stored at -80°C . TAG, cholesterol and glucose were measured with an ILAB 600 clinical chemistry analyser (Instrumentation Laboratory, Warrington, UK) using enzyme-based colorimetric tests supplied by Instrumentation Laboratory. NEFA were analysed as above using a kit supplied by Alpha Laboratories (Eastleigh, UK). All samples for each subject were analysed within a single batch and the inter-assay CV was less than 4% for all analyses.

Plasma insulin was measured using a specific ELISA (Dako Ltd, High Wycombe, UK). Total nitrite levels (surrogate marker of bioavailable NO) were determined using a commercially available kit following reduction of nitrate to nitrite (ACTIVE MOTIF, Rixensart, Belgium). The inter-assay CV for these assays was less than 6%.

Statistical analysis

Data were analysed using SPSS version 15.0 (SPSS, Inc., Chicago, IL, USA). Results are presented in the table as median values with their interquartile range and in the figure as mean values with their standard errors. The plasma metabolites in the baseline (0 h) and 4 h postprandial samples were analysed using a two-factor-mixed ANOVA (with time and group as the within and between factors, respectively). The flux values for the microvascular responses to ACh and SNP measured at baseline (0 h), 2 h and 4 h after the test meal in the younger and the older men (Fig. 1) were analysed using a three-factor-mixed ANOVA (two within-subject factors (time and flux units measured as the cumulative charge increased from 0 to 8000 C) and one between-subjects factor (male group)).

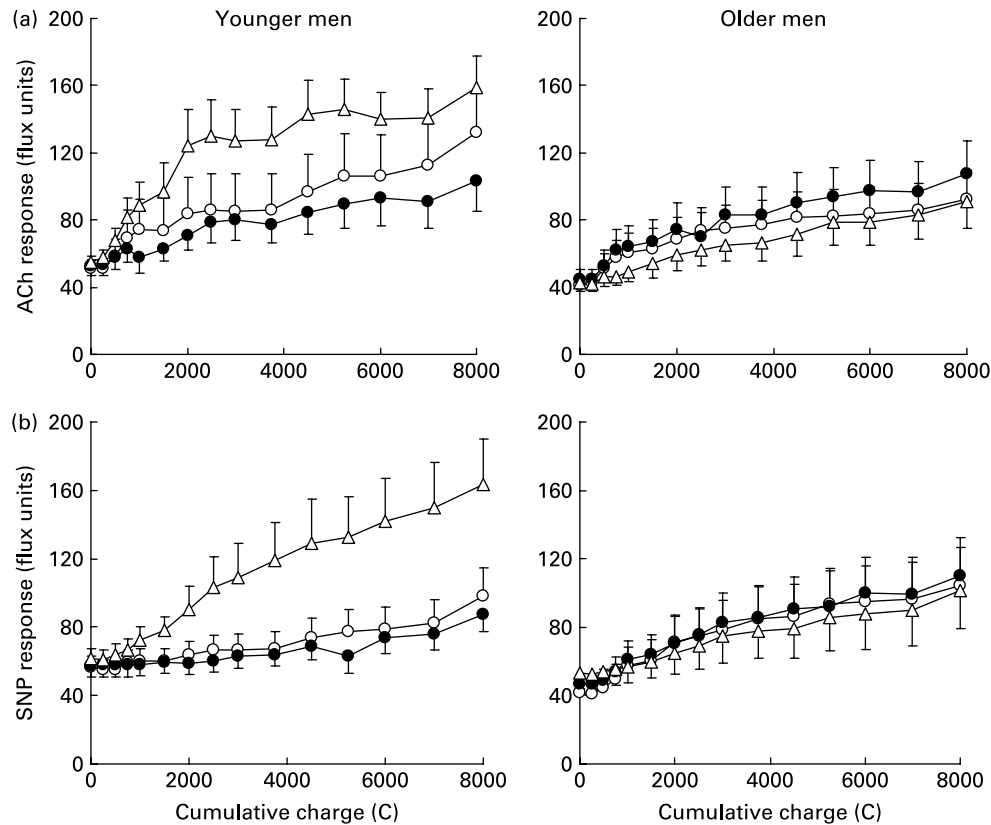


Fig. 1. Baseline and postprandial vascular responses to (a) acetylcholine (ACh) and (b) sodium nitroprusside (SNP) in the younger and older male groups. The flux units measured at baseline (○), 2 h (●) and 4 h (△) were plotted *v.* cumulative charge. Values are means for the twelve younger and twelve older men, with their standard errors represented by vertical bars. For the younger men, there was a significant interaction between the time of the vascular reactivity measurement and the magnitude of the flux units for the vasodilatory responses to ACh ($P=0.041$) and SNP ($P=0.021$). There was also a significant difference in the ACh ($P=0.006$) and SNP ($P=0.05$) vasodilatory responses to the test meal at 4 h between the two groups of men.

Simple correlations (Spearman's ρ) were performed to determine associations between age, BMI, plasma metabolites and the area under the curve (AUC) calculated using the trapezoidal rule for the vascular reactivity measurements at baseline, 2 and 4 h. Stepwise multiple linear regression was performed using P -in of 0.05 and P -out of 0.01 in order to establish the independent associations between the AUC for the vascular reactivity measurements and age, BMI and plasma metabolites. All data were checked for normality and log transformed where necessary before statistical analysis. These data included TAG, NEFA and insulin. Values of $P \leq 0.05$ were taken as significant.

Results

Plasma metabolites

Baseline and postprandial lipid, glucose, insulin and total nitrite concentrations in the two groups of men are shown in Table 1. Baseline metabolite concentrations were not significantly different between the younger and the older men, although the expected trend towards higher levels in the older participants was generally observed.

Compared with baseline concentrations, there was a significant increase in TAG ($P < 0.001$), total nitrite ($P = 0.022$) and NEFA ($P = 0.022$) concentrations at 4 h in the younger men. In addition, postprandial insulin concentrations were shown to

be lower than those at baseline ($P = 0.021$). In the older men, postprandial TAG ($P < 0.001$) and nitrite ($P = 0.045$) concentrations were significantly greater than baseline concentrations (Table 1).

The mixed factor ANOVA showed a difference in the NEFA concentrations between the male groups ($P = 0.010$), with the 4 h concentration shown to be significantly greater in the younger compared with the older men ($P = 0.005$; Table 1). Postprandial TAG, cholesterol, glucose, insulin and total nitrite concentrations were similar in both groups of men, although again the trends towards higher levels were evident in the older men.

Microvascular responses to acetylcholine and sodium nitroprusside at baseline, 2 h and 4 h

Baseline (0 h), 2 h and 4 h skin perfusion responses to ACh and SNP in the younger and the older men are shown in Fig. 1. These data are presented as flux units *v.* cumulative charge (0–8000 C); as the charge increases, there is an increase in drug delivery leading to a greater skin perfusion (flux units).

Although not reaching statistical significance, there was a tendency for the basal measurement of skin perfusion (i.e. flux units measured at 0 h, with 0 C so there is no SNP- or ACh-mediated activation) to be higher (33%) in the younger compared with the older men ($P = 0.107$).

Table 1. Lipid, glucose, insulin and total nitrite concentrations before and 4 h after the fish oil-enriched meal

(Median values with their interquartile range (IQR) for the twelve younger and twelve older men)

	Baseline (0 h)		Postprandial (4 h)		P
	Median	IQR	Median	IQR	
Younger men					
TAG (mmol/l)	1.00	0.79–1.22	1.95	1.02–3.12	<0.001
NEFA (μ mol/l)	280.1	215.8–368.1	450.5	299.1–538.2	0.022
Cholesterol (mmol/l)	5.18	4.03–5.38	5.17	4.24–5.55	0.083
Glucose (mmol/l)	5.74	2.50–6.27	5.44	4.93–6.24	0.625
Insulin (pmol/l)	18.3	11.4–29.9	14.3	7.6–20.5	0.021
Total nitrite (μ mol/l)	29.1	23.7–41.2	40.7	30.7–46.2	0.022
Older men					
TAG (mmol/l)	1.26	0.86–1.71	2.83	1.53–3.20	<0.001
NEFA (μ mol/l)	220.8	193.8–310.2	266.3*	215.9–352.2	0.500
Cholesterol (mmol/l)	5.36	4.46–6.47	5.54	4.60–6.80	0.118
Glucose (mmol/l)	5.54	5.25–5.90	5.25	4.97–5.76	0.353
Insulin (pmol/l)	25.2	19.0–33.8	19.8	11.2–38.7	0.101
Total nitrite (μ mol/l)	36.3	28.4–39.6	50.1	34.5–63.7	0.045

Values were significantly different from the 4 h NEFA concentration of the younger men: * $P=0.005$.

The mixed factor ANOVA revealed an interaction between the male group, the time of the vascular reactivity measurement after the test meal and the magnitude of the flux units in response to ACh ($P=0.037$) and SNP ($P=0.025$), indicating a difference in the vascular reactivity measurements between the younger and older men. In the younger male group, there was a significant interaction between the time of the vascular reactivity measurement after the test meal and the magnitude of the flux units measured in response to ACh ($P=0.041$) and SNP ($P=0.021$). In particular, the vasodilatory response to ACh and SNP was significantly greater at 4 h after the meal compared with the baseline (ACh response, $P=0.038$ and SNP response, $P=0.020$) and 2 h (ACh response, $P=0.012$ and SNP response, $P=0.010$) measurements (flux units measured at the end of the vascular reactivity measurement (i.e. at 8000 C) were on average 35% (ACh) and 75% (SNP) greater at 4 h compared with the baseline and 2 h measurements, respectively). Differences were not observed in the vasodilatory responses to ACh or SNP at baseline, 2 or 4 h after the meal in the older men.

Although the vascular reactivity measurements were similar in the two groups of men at baseline and 2 h, a significantly greater vasodilatory response to ACh ($P=0.006$) and SNP ($P=0.05$) was observed at 4 h in the younger compared with the older men.

Simple correlations and regression analysis

In bivariate Spearman's correlation analysis, age was shown to be negatively correlated ($r_s = -0.541$, $P=0.006$) with the basal measurement of skin perfusion. Furthermore, age and to a lesser extent BMI were shown to be negatively correlated with the AUC for the postprandial vasodilatory responses to ACh (Age $r_s = -0.656$, $P=0.001$ and BMI $r_s = -0.461$, $P=0.047$) and only age with the AUC for the postprandial vasodilatory response to SNP ($r_s = -0.549$, $P=0.005$) assessed at 4 h after the test meal. Of the plasma metabolites measured, only postprandial NEFA concentrations were shown to be positively correlated with the AUC for the

postprandial vasodilatory response to SNP at 4 h ($r_s = 0.437$, $P=0.033$), with a strong trend for a correlation with the AUC for the postprandial vasodilatory response to ACh at 4 h ($r_s = 0.399$, $P=0.053$).

In stepwise multiple regression analysis, age explained 18% of the variation in the basal measurement of skin perfusion (standardised coefficient -0.471 , $P=0.042$). Furthermore, age explained 54% of the variation in the AUC for the 4 h vasodilatory response to ACh (standardised coefficient -0.755 , $P=0.001$) and 28% of the variation in the AUC for the 4 h vasodilatory response to SNP (standardised coefficient -0.569 , $P=0.014$).

Discussion

Reduced vascular reactivity is becoming increasingly associated with the accelerated atherogenesis and essential hypertension, and serves as a marker for general vascular dysfunction and CVD risk. As a result, the importance of gaining an understanding of the physiological and lifestyle determinants of vascular tone is emerging as a priority area for CVD research.

In view of the positive effects of long chain *n*-3 PUFA supplementation on vascular reactivity measured in the fasting state, a (limited) number of studies have determined the acute impact of fish and/or fish oils on postprandial vascular reactivity^(13–15). Given that individuals consuming regular meals spend 18–20 h per day in the postprandial state, investigations of physiological and lifestyle determinants of non-fasting vascular function are a topic of recent research interest. In the present study, we found a single meal enriched in 5.4 g of DHA and EPA, equivalent to two portions of oily fish, improved both endothelial-dependent (ACh) and endothelial-independent (SNP) vasodilation in our younger male group at 4 h after the meal. No impact of the fish oil-enriched meal was observed on the postprandial vascular response in our older male participants. Multiple regression analysis revealed that age was a predominant influence on both the

postprandial vasodilatory response to ACh, and to a lesser extent SNP, 4 h after the meal.

Vascular tone is complexly regulated by neural, humoral and a range of vasomodulatory factors produced by the cells within the vessel wall itself, with endothelial-derived NO recognised to be a particularly important modulator⁽⁴⁾. A number of mechanisms to explain the beneficial effects of fish oils on endothelial function have been derived from chronic supplementation studies in both human subjects and animals. Observations suggest that fish oil fatty acids either (a) increase the ability of the endothelium to produce NO (via effects on endothelial NO synthase enzyme activity), (b) decrease NO degradation and/or (c) increase the responsiveness of the smooth muscle cell layer to NO^(10,20). Therefore, it appears that fish oil fatty acids could influence both endothelial-dependent (ACh) and endothelial-independent (SNP) vasodilatory mechanisms. Since the present study was designed to examine the impact of age on response to a standard test meal, and therefore did not include a reference non-fish oil-containing meal, definite conclusions regarding the impact of EPA and DHA on vascular reactivity cannot be made.

Ageing is associated with progressive endothelial dysfunction, with endothelial-dependent vasodilation reported to decline by 0.21 % per year in healthy men over 40 years of age with little impact of ageing on endothelial-independent vasodilation⁽²¹⁾. Proposed mechanisms for the impairment in endothelial function with ageing include a decreased release of NO and/or increased rate of degradation of NO induced by oxidative stress^(2,7). In the present study, consumption of the test meal had little impact on the vascular reactivity measurements in our older age group, even though total nitrite concentrations were significantly elevated at 4 h after the meal. Surprisingly, total nitrite concentrations in the older men were comparable with those of the younger men at both the baseline and postprandial time points. Since total nitrite levels have been regarded as an index of endothelial NO formation *in vivo*⁽²²⁾, the present data suggest that NO production was not impaired or that there was not an excessive degradation of NO in our older men. As with the findings of Skilton *et al.*⁽⁸⁾, multiple regression analysis indicated that increasing age was associated with the changes in postprandial endothelial-dependent vasodilation. In the present study, age was also shown to be associated with endothelial-independent vasodilation, a measure of smooth muscle reactivity. As previously suggested in the literature^(23,24), it is possible that structural changes in the vascular wall with ageing may have slowed down the rate of diffusion of NO and NEFA enriched in the meal fatty acids to the smooth muscle cell layer in our older age group. In support of this mechanism, studies in isolated aortic rings from ageing rats have shown removal of the endothelial cell layer to enhance the vaso-relaxing effects of EPA and DHA⁽²⁵⁾.

Only one previous study has examined the impact of ageing on postprandial vascular reactivity⁽⁸⁾ with older men showing a tendency for a lower forearm blood flow 3 and 6 h, following a SFA-rich meal compared with the younger men. In agreement with Skilton *et al.*⁽⁸⁾, measurements of vascular reactivity in the fasting state were not significantly different between our two groups of men with differences only observed after the consumption of a high-fat meal.

Interestingly, of all of the parameters measured, only postprandial NEFA concentrations were significantly elevated in the younger compared with the older men and also shown to be correlated with the magnitude of the vasodilatory response to SNP at 4 h. It is therefore possible that a greater exposure of the endothelium to NEFA enriched in fish oil may be mediating these positive effects on the vascular wall, and in particular the smooth muscle cell layer in our younger age group.

In conclusion, the present study has shown the ingestion of a test meal containing fish oil to improve postprandial vascular reactivity at 4 h after the meal in the younger men only. The lack of responsiveness of the microcirculation in the older men to meal ingestion is an interesting observation and could be reflective of more widespread endothelial dysfunction. However, further work is warranted to determine the mechanism(s) responsible for the lack of effect of meal ingestion and in particular fish oil fatty acids on postprandial vascular reactivity in older men.

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