Higher body fat percentage is associated with enhanced temperature perception in NAFLD: results from the randomised WELCOME* trial

*WELCOME = **W**essex **E**valuation of fatty **L**iver and **C**ardiovascular markers in NAFLD with **OM**acor th**E**rapy trial)

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Abstract 248

Aims/hypothesis The effect of omega-3 fatty acid treatment on temperature perception as a sensory nerve function modality is uncertain. In patients with non-alcoholic fatty liver disease (NAFLD) both with and without type 2 diabetes (T2DM), we: a) tested whether 15-18 months treatment with 4 g/day of docosahexaenoic+eicosapentaenoic acid (DHA+EPA) improved hot (HPT) and cold (CPT) temperature perception thresholds and b) explored factors associated with HPT and CPT, in a randomised, double-blind, placebo-controlled trial.

Methods The effect of treatment (n=44) on HPT, CPT and TPI (difference between HPT and CPT) was measured at the big toe in 90 individuals without neuropathy (T2DM n=30). Treatment-effects and the independence of associations were testing by regression modelling.

Results Mean±SD age was 50.9±10.6 years. In men (n=53) and women (n=37), HPTs (^{o}C) were 46.1±5.1 and 43.1±6.4 (p=0.02), CPTs (^{o}C) were 22.7±3.4 and 24.5±3.6 (p=0.07) and TPIs (^{o}C) were 23.4±7.4 and 18.7±9.5 (p=0.008), respectively. In univariate analyses, total body fat percentage (measured by dual X-ray absorptiometry) was associated with HPT (r=-0.36 p=0.001), CPT (r=0.35 p=0.001) and TPI (r=0.39 p=0.0001). In multivariable-adjusted regression models, adjusting for age, sex and other potential confounders, only body fat percentage was independently associated with HPT, CPT or TPI (p=0.006, p=0.006 and TPI p=0.002), respectively. DHA+EPA treatment did not modify HPT, CPT or TPI (p=0.93, 0.44, 0.67), respectively.

Conclusions/interpretation Higher body fat percentage is associated with enhanced temperature perception. There was no benefit of treatment with high dose omega-3 fatty acids on the thresholds to detect hot or cold stimuli.

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Abbreviations List

- ACEI- Angiotensin converting enzyme inhibitors
- CPT – Cold temperature Perception Threshold
- CV - Coefficient of Variation
- DXA - Dual energy X-ray absorptiometry
- DHA - Docosahexaenoic Acid
- EPA - Eicosapentaenoic Acid
- HPT – Hot temperature Perception Threshold
- IR - Insulin Resistance
- MF - Maximum Flux
- MF/RF - The ratio of Maximum Flux (MF) to Resting Flux (RF)
- MR - Microvascular Reactivity
- MRI - Magnetic Resonance Imaging
- MRS - Magnetic Resonance Spectroscopy
- NAFLD - Non-Alcoholic Fatty Liver Disease
- PU - (arbitrary) Perfusion Units
- QST - Quantitative sensory testing
- RF - Resting Flux
- TPI - Temperature Perception Index
- VPT - Vibration Perception Threshold

Introduction

Small sensory nerve fibre neuropathies (SFSN) give rise to the symptoms of impaired nerve sensitivity and neuropathic pain which affect over 90% of people with type 1 and type 2 diabetes [1, 2] and are the main initiating factors for foot ulceration and lower limb amputation in people with diabetes [3]. The mechanisms underlying SFSN and neuropathic pain are not well understood but may include metabolic factors such as elevated intracellular glucose levels in both vascular and neural tissues which can lead to increased oxidative stress and/or microvascular impairment and reduced nerve blood flow [4, 5].

Quantitative sensory testing (QST) is a method for assessing the somatosensory nervous system and is routinely used in the clinical assessment of large and small sensory nerve fibre function [6, 7]. We have recently shown using QST of large myelinated nerve function that both ageing and a measure of microvascular reactivity were independently associated with altered vibration perception threshold (VPT) at 125Hz in a high risk patient group without evidence of overt peripheral neuropathy [8]. QST has also been used to study functional deficits in small fibre neuropathies, with temperature perception thresholds being one of the more widely employed tests [9-11]. Such tests provide an indirect but reproducible assessment of human small nerve function [12, 13].

There are as yet no treatments that prevent or reverse painful diabetic neuropathy. Vasodilator sprays may improve nerve blood flow [14] and alpha lipoic acid [15] (as a sulphur containing product derived from the eight carbon atom fatty acid octanoic acid) has been shown to give moderate pain relief in clinical trials, with fewer side effects than opioids and antidepressants [16]. Treatment with high-dose long-chain polyunsaturated omega-3 fatty acids such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) has been shown to improve vibration perception and clinical symptoms of neuropathy in patients with type 2 diabetes [17] and to improve endothelial function in dyslipidaemia, overweight men [18], as well as offering neuroprotection by increasing nerve blood flow in an experimental model of diabetic neuropathy [19]. However, the benefit of long chain omega-3 fatty acids in patients at risk of, but without peripheral neuropathy, remains uncertain.

In a pre-specified sub-study of the **W**essex **E**valuation of fatty **L**iver and **C**ardiovascular markers in Non Alcoholic Fatty Liver Disease (NAFLD) with **OM**acor th**E**rapy (WELCOME) trial (www.clinicaltrials.gov registration number NCT00760513) [20, 21] (a randomised double blind placebo-controlled trial), we tested the effect of DHA+EPA treatment on hot and cold temperature perception thresholds. Our overall aim was to test the hypothesis that the highest licensed dose of DHA+EPA omega-3 fatty acid treatment (4 g/day as ethyl esters) over a minimum of 15 months and a maximum of 18 months had beneficial effects on small sensory nerve function in high risk patients for neuropathy, who had insulin resistance and NAFLD (with or without type 2 diabetes).

Methods

Study design

The participants formed part of the WELCOME study (REC: 08/H0502/165) [20, 21]. One hundred and five individuals were recruited to the study (see the supplementary material for the Consort Diagram and testing the effect of the intervention on this secondary end point). Subjects were excluded from the current analysis if they had evidence of distal peripheral neuropathy in their feet, as suggested by their failure to detect a 10 g monofilament [22] (n=4), or if they had evidence of diabetic eye disease at retinal screening (n=0). Baseline and end of study thermal thresholds were obtained on ninety individuals (53 men and 37 women (mean age of 50.9 ($\pm$10.6) y) of whom 44 were on active treatment and 46 on placebo. Participants gave their informed written consent. Biochemical and anthropometric measurements have been described previously [8].

Peripheral neurological and microvascular function testing

All tests were conducted in a temperature controlled room (22 - 24^{o}C) as described previously [8]. Participants refrained from caffeine-containing drinks, smoking and exercise for ≥2 h prior to testing. Measurements were made at baseline and end of study.

Temperature perception testing Hot (HPT) and cold (CPT) temperature perception thresholds were measured on the pulp of the left big toe using a thermal aesthesiometer (HVLab Diagnostics Instruments) [23] (see supplementary material for details). A threshold perception 'index' (TPI) was constructed for each individual, calculated as the arithmetic difference between HPT and CPT (^{o}C) measured at baseline [24].

Vibrotactile perception testing Vibration perception thresholds (VPTs) at 125Hz were measured on the pulp of the left big toe as described previously [8] (see supplementary material for details).

Microvascular function Cutaneous microvascular blood flow was assessed on the ventral surface of the non-dominant forearm arm using laser Doppler fluximetry (MoorVMS LDF2, Moor Instruments Ltd, Axminster, UK) at rest (RF) and during the dilator response to transient ischaemia (180 mmHg for 3 min; MF) [8]. (see supplementary material for details).

Statistical analysis

Statistical analyses were undertaken using IBM SPSS Statistics 21.0 (IBM United Kingdom Limited, UK). Pearson and Spearman rank correlation coefficients were used to investigate associations between normally and non-normally distributed variables, respectively. In all cases a value of $p<0.05$ was taken to indicate significance. Multivariable linear regression modelling was used to test the independence of associations between baseline variables and baseline measurements of HPT, CPT and TPI. Explanatory factors that were included in the regression models included, age, sex, diabetes (yes/no) (or HOMA-IR), measures of body fat and MF/RF. In addition analyses we also tested for effects of medications and skin temperature. We also tested the effects of DHA+EPA treatment on TPI and both HPT and CPT using multivariable linear regression modelling. For all three outcomes of interest the outcome represented the 'change' variable i.e. the difference between the end of study measurement and the baseline study measurement. For each separate regression model, there was also adjustment for the baseline measurement of the respective outcome variable in question. Each of these models included DHA+EPA treatment as a dichotomous exposure variable age, sex, the temperature threshold variable at baseline (of the respective outcome variable) and additional relevant exposures of interest, selected from the univariable analyses (including total body fat %, MF/RF, and diabetes status (y/n) or insulin resistance (HOMA-IR).

Results

Characteristics of the group

Table 1 shows the baseline characteristics of the participants without overt neuropathy or microvascular disease. Of the participants, 35 were treated with statins, 32 with antihypertensive drugs, 7 with fibrates, 18 with angiotensin converting enzyme inhibitors (ACEI), 24 with metformin and 13 with antidepressants.

Effect of the omega-3 polyunsaturated fatty acid intervention on temperature perception thresholds

Individual baseline and end of study measurements for HPT, CPT and TPI for participants are shown in **Figure 1. Table 2** shows the results of multivariable regression modelling testing the effects of the DHA+EPA intervention on the change in HPT, CPT and TPI (i.e. the change between the end and start of the study) and shows no effect of the treatment on each of the outcomes of interest. Swapping HOMA-IR for diabetes status had little effect on the models.

Associations between hot and cold temperature perception thresholds and cardiovascular and metabolic risk factors at baseline

In univariate analyses (see supplementary material for table of univariate analyses showing associations between hot and cold temperature perception thresholds and cardiovascular and metabolic risk factors at baseline), both HPT and CPT were associated with sex (r=-0.252 p=0.017 and r=0.244 p=0.021, respectively), with women being more sensitive to the thermal stimuli than men. HPTs and CPTs were associated with total body fat percentage and subcutaneous abdominal fat quantity. The associations between HPT and CPT measured at the big toe and percentage body fat were independent of skin temperature (mean skin temperature measured at the big toe prior to testing was 28.2 ± 2.5^{o}C (n=90) and were not affected by adjustment for skin temperature. Scatter plots for the association between HPT and CPT and body fat are shown in **Figure 2**. The direction of the correlations shows that temperature perception (or the ability to detect the stimulus after a smaller temperature change) increases with increasing total body fat percentage. TPI showed a similar association with total body fat percentage (r=0.39, p=0.0001) (**Figure 2**) with the direction of

correlation showing a reduction in TPI with increasing body fat. Similar trends were seen at the left middle finger (data not shown).

There was no significant association between HPT and CPT and measures of glycaemic status (fasting glucose concentration or HbA1c, subjects with or without diabetes) or insulin resistance (HOMA-IR, subjects without diabetes. In individuals with diabetes (n=30), HPT was associated with the duration of diabetes (r=0.471 p=0.009), but CPT was not (r=-0.267 p=0.154).

HPT was associated with CPT (r=-0.670, p<0.0001), a finding consistent with that in a cohort of 300 healthy students [25]. Temperature perception was also associated with vibration perception threshold (VPT) measured at 125Hz at the big toe [8] (HPT r=0.326, p=0.002; CPT r=-0.211, p=0.046).

Using regression modelling, we explored the independence of associations between HPT, CPT and TPI and each of the variables that were shown to be associated with these outcomes in the univariate analyses. HPT, CPT and TPI were the respective outcomes in these separate regression models (**Table 3**). These multivariable models showed that the exposure variables in the models explained 21% of the variance in HPT, 18% in CPT and 23% of the variance in TPI. Of note, in each of these regression models there was a strong independent association between total body fat percentage and HPT, CPT and TPI. Swapping HOMA-IR for diabetes status had little effect on each regression model (data not shown). Use of statins, fibrates, metformin, antidepressants or all antihypertensive drugs (including calcium antagonists and ACEI) was not independently associated with thermal perception thresholds when each factor was added to the model as a dichotomous exposure variable (data not shown).

Discussion

Our novel results show no benefit from DHA+EPA treatment on hot or cold temperature perception thresholds, in patients with NAFLD, both with and without diabetes. We have examined the effects of the highest licensed dose of omega-3 fatty acids (4 g/day as Omacor/Lovaza) on measures of small sensory nerve fibre function in a randomised double blind placebo controlled trial lasting a minimum of 15 months and a maximum of 18 months in subjects at risk of developing diabetic neuropathy and neuropathic pain. To our knowledge, our study is the first randomised double blind placebo controlled trial to examine the effects of DHA+EPA on temperature perception. We also show for the first time that hot and cold thermal perception thresholds were strongly and independently associated with total body fat percentage (but not with liver fat or with glycaemic control) in men and women.

We found no association between hot or cold temperature perception thresholds and measures of insulin sensitivity or HbA1c in these patients who were at risk of neuropathy. Our findings are consistent with the results of a study of 156 individuals with peripheral neuropathy where there was no association between neuropathy and glycaemic control [26]. Elevated hot thresholds have been shown to be the most frequent sensory deficit in individuals with diabetes and hot thresholds have been shown to be correlated with glycaemic control in some [9, 27] but not all [28, 29] studies. In those individuals with diabetes, HPT was associated with duration of diabetes, possibly due to increased oxidative stress, nerve damage or reducing nerve fibre densities that are early events in people with diabetes [30].

We also observed correlations between hot and cold temperature perception thresholds and total body fat percentage measured by DXA, and with abdominal subcutaneous fat assessed using MRI. Surprisingly, the directions of these associations indicate that thermal sensitivity (or the ability to detect the stimulus with a small temperature change) *increased* as the proportion of body fat increased. Importantly, these associations were independent of sex and of age [31]. Temperature thresholds depend on the contact area between the skin and the warm or cool surface with greater sensitivity when more receptors are excited by a larger contact area [32]. It is possible that increased subcutaneous fat resulted in an

increased area of contact between the surface of the thermal aesthesiometer and the big toe but it was not possible to adjust for the size of the big toe in this study. The changes in temperature perception thresholds may alternatively be associated with skin thickness and thermal conduction and/or nerve and tissue blood flow but investigation of these factors was beyond the scope of the present study. Obesity is characterized by a low-grade chronic inflammatory state which is known to modulate pain and people with chronic pain tend to have a greater total fat mass [33]. Thus it is possible that the increase in temperature sensitivity with increasing body fat percentage that we observe in our cohort may to be due to a change in small sensory nerve fibre function modulated by an increased release of pro-inflammatory cytokines or adipokines from adipose tissue.

The current study extends previous data suggesting that the functions of both small and large sensory fibres are impaired in neurologically asymptomatic individuals both with and without diabetes. The temperature perception thresholds to hot and cold stimuli associated with activation of small sensory afferent A-delta and C-nerve fibres that we measured at the big toe were strongly correlated with large sensory nerve fibre VPT measured at 125Hz [8]. Large-fibre nerve dysfunction measured by VPT predicts foot ulceration, lower limb amputation, and mortality [26]. However, to what extent assessment of small fibre function using QST may represent an additional screening tool to monitor disease progression and therapeutic impact remains unclear [35].

Limitations

QSTs are psychophysical tests and their use as a diagnostic tool alone or in combination with other more direct tests much debated [35]. We cannot overlook the fact that there are a number of factors that may affect temperature perception thresholds which we have been unable to measure, including alertness and attention, integration of sensory information from the stimulated skin area and skin thickness [25]. Our results provided no indication of an improvement with the intervention (**Table 2**). A retrospective power calculation based on a sample size of n=90 with n=44 in the treatment arm of the trial shows that we had 84% power to detect a 3.5^{o}C temperature difference in HPT between baseline and the end of study with DHA+EPA treatment. That we observed only a 1^{o}C mean temperature difference in HPT between baseline and end of study in the treatment arm, strongly suggests that

there was no effect of the intervention on this parameter but we would have needed a 10 times bigger sample size to prove there was no effect of treatment. Additionally, with the very low CV (~6%) of the temperature perception threshold measurement techniques, this gives us further confidence in the validity of the findings. There was a lack of worsening of temperature perception thresholds in the placebo arm of the study. This is consistent with our previous study on large sensory nerve fibre thresholds [8] and that of others [36] and underscores the challenges of sensory nerve fibre testing studies in patients with well-controlled diabetes. Finally, we cannot be sure of the effect of duration of diabetes on temperature perception in our cohort, as identification of the date of onset of type 2 diabetes is often imprecise.

Conclusions

In summary, treatment with the highest licensed dose of DHA+EPA (as Omacor/Lovaza) did not modify small nerve fibre-mediated temperature perception in patients with NAFLD with and without diabetes. In this high-risk patient group, without evidence of overt peripheral neuropathy, percentage body fat was independently associated with HPT and CPT which were related more to a measure of overall body fatness than to metabolic risk factors.

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Duality of Interest

PCC serves on the Clinical Advisory Board of Pronova Biopharma and has acted as a consultant to Amarin. None of the other authors has any disclosures.

Contribution Statement

All the named authors fulfil all three International Committee of Medical Journal Editors (ICMJE) uniform requirements for authorship of this manuscript. KMcC, CDB, GFC and MJG made a substantial contribution to the conception and design of the study. KMcC, ES and LB made a substantial contribution to the acquisition of the data. CDB, GFC, KMcC, PCC and MJG conducted the analysis and interpretation of data. CDB and GFC drafted the manuscript, PCC, MJG, ES, and LB helped to revise it critically for important intellectual content. All the contributing authors approved the final version to be published. CDB is responsible for the integrity of the work as a whole and is the guarantor of this work.

Figure legends

Figure 1. Effect of 15-18 months treatment with docosahexanoic acid+eicosapentanoic acid (DHA+EPA) on hot (HPT) and cold (CPT) temperature perception threshold and temperature perception index (TPI) (HPT-CPT) measured at the big toe. Figure a, b and c represent the placebo group and d, e and f represent the treatment group.

Figure 2. Scatter plots showing the relationship between a. baseline hot (HPT) and cold (CPT) temperature perception thresholds and b. temperature perception index (TPI) (HPT-CPT) measured at the big toe and total body fat percent measured using DXA.

1 **Table 1. Baseline characteristics according to diabetes status and randomisation group**.

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	No Diabetes	Diabetes	*P* value	Randomised to Placebo	Randomised to DHA+EPA	*P* value
	n=60	n=30		n= 46	n=44	
Sex (M/F)	35/25	18/12		31/15	22/22	
Age (y)	51.5±10.7	49.8±10.6	0.69	54.1±9.6	47.6±10.7	0.003
BMI (kg/m^2)	32.1(4.8)	33.4(6.0)	0.03	31.9(5.2)	32.4(7.1)	0.09
Duration of Diabetes (y)		3(3.5)		6(5)	3(5)	0.45
Diabetes				16	14	
Current smoker	6	3	0.75	4	5	0.71
10 Year Cardiovascular Disease Risk(QRISK2, %)	9.7(7.5)	14.7(9.4)	0.02	12.5(8.3)	7.5(13.4)	0.15
SBP (mmHg)	137(16)	135(14)	0.61	137(17)	135(26)	1.0
Cholesterol (mmol/l)	4.8(1.4)	4.3(0.9)	0.01	4.5(1.4)	4.8(1.3)	0.06
Low-density lipoprotein Cholesterol (mmol/l)	2.9(0.9)	2.6(0.9)	0.16	2.7(1.0)	3.0(1.3)	0.14

High-density lipoprotein Cholesterol (mmol/l)	1.1(0.4)	1.0(0.2)	0.15	1.1(0.3)	1.0(0.4)	0.17
Cholesterol/HDL	5.0±1.5	4.7±1.5	0.49	4.5±1.4	5.3±1.6	0.11
Triacylglycerol (mmol/l)	1.8(1.3)	1.5(1.0)	0.56	1.5(1.0)	1.8(1.2)	0.55
Total body fat (DXA, %)	37.7±7.5	36.8±7.0	0.57	35.6±6.9	39.4±7.1	0.38
Subcutaneous Fat (MRI, %)	32.4± 9.9	31.5±10.7	0.68	29.9±9.4	34.4±10.2	0.14
Visceral Fat (MRI, %)	16.0±4.8	16.3± 5.2	0.83	16.8±4.6	15.4±5.2	0.30
Liver Fat (MRS, %)	23.3(21.8)	21.8(14.9)	0.95	21.7(20.1)	23.0(33.9)	0.44
NAFLD fibrosis score *	8.7(0.7)	9.0(0.9)	0.05	8.9(0.9)	8.7(1.0)	0.42
Insulin Resistance (HOMA-IR)	2.7(2.5)	4.3(4.1)	0.01	2.8(3.2)	2.9(3.2)	0.60
HbA_{1C} (%)	5.8(0.6)	7.7(1.8)	0.03	6.1(1.6)	5.9(1.2)	0.13
(mmol/mol)	42.8 (4.4)	56.9 (13.3)		45.0 (11.8)	43.6 (8.9)	
Microvascular reactivity (MF/RF)	4.2(1.5)	5.0(1.8)	0.33	4.3(2.0)	4.3(2.4)	1.0
Vibration Threshold (125Hz) (m.s-2)	3.7(5.6)	3.6(6.7)	0.48	5.5(6.5)	2.9(4.0)	0.03

Temperature perception threshold (°C)						
Hot	45.4(6.0)	46.7(5.6)	0.76	45.4(5.8)	43.8(5.7)	0.19
Cold	23.0(3.6)	24.4(3.4)	0.38	22.6(3.5)	24.8(3.9)	0.08

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2 Data are presented as mean±SD or median (IQR) (*liver fibrosis score, see reference [34]).

Table 2 Multivariable regression models showing the effects of DHA+EPA treatment on hot and cold temperature perception thresholds, and on the temperature perception index (TPI), adjusting for baseline measurement of each respective outcome, and key covariates and potential confounders as explanatory variables.

	Primary outcomes		
	Thermal perception threshold index (°C) Change from baseline to end of study	**Hot thermal perception threshold (HPT °C) Change from baseline to end of study**	**Cold thermal perception threshold (CPT °C) Change from baseline to end of study**
Independent variables	**Unstandardised B coefficient (95% CI) *p* value**	**Unstandardised B coefficient (95% CI) *p* value**	**Unstandardised B coefficient (95% CI) *p* value**
Treatment Docosahexanoic acid + Eicosapentanoic acid	-0.660 (-3.680, 2.359) 0.665	-0.091 (-2.25,2.07) 0.93	0.575 (-0.88,2.03) 0.44
Age (y)	-0.014 (-0.159, 0.130) 0.845	0.045 (-0.059,0.148) 0.39	0.053 (-0.02,0.12) 0.13
Male sex	3.122 (-1.823, 8.068) 0.213	1.31 (-2.22,4.85) 0.46	-1.91 (-4.27,0.46) 0.11
Diabetes status (y/n)	0.007 (-2.909, 2.924) 0.996	-0.041 (-2.13,2.05) 0.969	-0.097(-1.51,1.31) 0.89
Temperature perception threshold/index at baseline	-0.392 (-0.573, -0.212) <0.0001	-0.451 (-0.64,-0.26) <0.0001	-0.391 (-0.60,-0.19) <0.0001
MF/RF	-0.178 (-1.113, 0.758) 0.707	-0.213 (-0.88,0.46) 0.53	0.013 (-0.43,0.46) 0.057

Total body fat DXA (%)	-0.167 (-0.519, 0.185) 0.348	-0.077 (-0.33,0.17) 0.54	0.11 (-0.06,0.28) 0.19

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Table 3 Multivariable regression models with the hot (HPT) and cold (CPT) temperature perception thresholds and temperature perception index (TPI) as the outcomes, and key covariates and potential confounders as explanatory variables.

	Primary outcomes		
	Temperature perception threshold index (°C) at baseline	**Hot temperature perception threshold (HPT °C) at baseline**	**Cold temperature perception threshold (CPT °C) at baseline**
Independent variables	Unstandardised B coefficient (95% CI) p value	Unstandardised B coefficient (95% CI) p value	Unstandardised B coefficient (95% CI) p value
Age (y)	0.129 (-0.033, 0.291) 0.116	0.086 (-0.024,0.197) 0.125	-0.043 (-0.112,0.026) 0.218
Male sex	2.7 (-3.5218, 8.619) 0.367	1.671 (-2.376,5.718) 0.414	-1.030 (-3.552,1.452) 0.419
MF/RF	-1.04 (-2.141, 0.060) 0.064	-0.775 (-1.527,-0.022) 0.044	0.266 (-0.203,0.735) 0.263
Diabetes status (y/n)	-0.234 (-3.739, 3.270) 0.895	0.497 (-1.899,2.893) 0.681	0.731 (-0.762,2.225) 0.333
Total body fat DEXA (%)	-0.622 (-1.016, -0.227) 0.002	-0.383 (-0.653,-0.113) 0.006	0.238 (0.070,0.406) 0.006

Multivariable regression models for all subjects completing the randomised double blind placebo-controlled trial testing the effect of DHA+EPA treatment on each of the primary outcomes. Placebo group n=46, treatment group n=44. Each regression model was adjusted for age, sex,

outcome variable value at baseline, plus diabetes status (yes/no), a measure of microvascular dilator capacity (MF/RF) and total body fat % DXA). For the models TPI $R^2 = 0.231$; adjusted $R^2 = 0.186$ (p=0.001). HPT $R^2 = 0.21$; adjusted $R^2 = 0.166$ (p=0.001). CPT $R^2 = 0.182$; adjusted $R^2 = 0.133$ (p=0.004)

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