British Journal of Nutrition (2010), **103**, 243–248
doi:10.1017/S000711450999153X

Dose-dependent increases in heart rate variability and arterial compliance in overweight and obese adults with DHA-rich fish oil supplementation

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(Received 16 February 2009 – Revised 14 July 2009 – Accepted 15 July 2009 – First published online 7 August 2009)

Heart rate (HR) variability and large arterial compliance can be improved using fish oils. DHA, a component of fish oil, has cardiovascular health benefits, but its effect on HR variability (HRV) and arterial compliance is yet to be quantified. Sixty-seven overweight or obese adults (thirty-six males and thirty-one females; 53 (SEM 2) year; BMI 31·7 (SEM 1·1) kg/m^2) were randomly allocated to consume either 6 g/d sunola oil (control; *n* 17), fish oil (260 mg DHA + 60 mg EPA per g) at doses of 2 g/d (*n* 16), 4 g/d (*n* 17) or 6 g/d (*n* 17). Blood pressure, HR and compliance of large and small arteries were measured while supine at baseline and after 12 weeks in all participants, and HRV was assessed in a subgroup of forty-six participants. There was no effect of fish oil on blood pressure, small artery compliance or HR. However, the low frequency:high frequency ratio of HRV decreased with increasing doses of fish oil (r −0·34, P=0·02), while large artery compliance increased (r 0·34, P=0·006). Moreover, the changes in these biomarkers were significantly correlated (r −0·31, P=0·04) and may reflect fish oil-induced improvements in arterial function and cardiac autonomic regulation.

***n*-3 PUFA: DHA: Cardiovascular health: Cardiac autonomic balance**

Elevated heart rate (HR) is a risk factor for cardiovascular death, particularly sudden death[1], while impaired HR variability (HRV) is an indicator of mortality risk both in patients suffering from heart disease[2] and in the general population[3]. Arterial compliance is also an independent risk factor for CVD[4] and may contribute to cardiovascular risk by contributing to a reduction in HRV as a result of baroreceptors in the walls of less compliant arteries, being less able to respond to changes in blood pressure and therefore provide less sensitive regulation of HRV.

HRV refers to the beat-to-beat alterations in HR thought to reflect changes in autonomic nervous system activity. In healthy individuals during rest, the electrocardiogram (ECG) displays periodic variation in R–R intervals. There are two important frequency components of HRV, high frequency (HF, 0·15–0·4 Hz) and low frequency (LF, 0·04–0·15 Hz). The HF component has been shown to reflect efferent parasympathetic activity (predominant at rest), whereas the LF component reflects sympathetic and parasympathetic interactions as well as baroreceptor activity. The LF:HF ratio of HRV therefore reflects the balance between the sympathetic and parasympathetic nervous activity known as sympathovagal balance. Depressed HRV has been identified as a cardiovascular risk factor and increases the mortality risk among patients with and without heart disease[2,3].

HRV and arterial compliance are known to be attenuated in people with elevated levels of TAG[5,6]. High TAG are also strongly associated with obesity and have been evaluated as a significant risk factor for CVD[7,8]. Obesity is an independent cardiovascular risk factor; however, the mechanism underlying this association remains unclear. Several causes for the relationship between obesity and CVD have been suggested, including that a reduction in HRV or impaired arterial compliance might be the means for the increased cardiovascular risk[9–14]. It remains unclear whether arterial compliance or HRV measures in obese people with elevated blood TAG are affected by long-chain *n*-3 (LC *n*-3) PUFA in a dose-dependant manner.

Supplementation of the diet with fish oil containing LC *n*-3 PUFA has previously been shown to reduce TAG and HR[15,16] and improve HRV[17–19]. Epidemiological evidence also suggests that HRV is improved in populations that have a higher intake of LC *n*-3 PUFA over a prolonged period[20]. The effects of LC *n*-3 PUFA on HR and HRV are likely to be attributable to increased parasympathetic activation[19]. We hypothesise that the latter could result from fish oil-mediated improvements in arterial compliance, thereby increasing baroreceptor sensitivity.

While the effects of LC *n*-3 PUFA on HR and HRV could reduce the risk of cardiovascular events, it is unclear what

Abbreviations: ECG, electrocardiogram; HF, high frequency; HR, heart rate; HRV, HR variability; LAC, large artery compliance; LC *n*-3, long-chain *n*-3; LF, low frequency.

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dose is needed to achieve these benefits. The purpose of the present study was to investigate the dose–response effects of LC *n*-3 PUFA on HR and HRV in order to better understand what dose is required to achieve benefit, and to determine whether some of the effects of LC *n*-3 PUFA on HR and HRV might be mediated by improvements in arterial compliance.

Materials and methods

A randomised, double-blind, placebo-controlled, parallel dose–response supplementation trial of 12 weeks duration was undertaken. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Human Research Ethics Committees of the University of Adelaide and the University of South Australia (Adelaide, Australia). Written informed consent was obtained from all subjects before commencement.

Participants

Seventy-five overweight adults (thirty-eight males and thirty-seven females; 53 (SEM 2) year; BMI 31·7 (SEM 1·1) kg/m^2) were recruited for the study. Participants taking lipid-lowering, blood-thinning or antihypertensive medication, fish oil supplements or consuming more than one serving of fish per week were excluded. Participants were all non-smokers and were instructed to maintain their habitual exercise levels.

Study design

Participants were block matched into four groups that were stratified according to fasting serum TAG concentration. The groups were then randomised to consume six 1 g oil capsules/d comprising either 0 (*n* 17), 2 (*n* 16), 4 (*n* 17) or 6 (*n* 17) × 1 g capsules of DHA-rich fish oil (NuMega Ingredients, Altona North, Vic, Australia) with the balance of the capsules made up of 1 g sunola oil (contents are specified in Table 1) capsules (NuMega Ingredients). The 2, 4 and 6 g/d doses of fish oil provided 0·52, 1·04 and 1·56 g DHA/d, respectively. Height and weight, HR, arterial compliance and blood pressure were measured at baseline and after 12 weeks after an overnight (10–12 h) fast, and blood was collected by venepuncture. Forty-nine participants who were willing to undertake an ECG were measured for HRV.

Assessment of erythrocyte fatty acid profiles

The relative proportions of *n*-3 PUFA in erythrocytes were determined as described previously, and these assessments with blood lipids been have reported elsewhere[21].

Resting heart rate, arterial compliance and blood pressure

Assessments of compliance in large (LAC) and small arteries were obtained using the HDI/Pulsewave CR-2000 Cardiovascular Profiling System (Hypertension Diagnostic, Inc., Eagan, MI, USA) following 10 min of rest in the supine position. An appropriate blood pressure cuff was placed about the subject's left upper arm, and a rigid plastic wrist support was placed on the subject's right wrist to minimise wrist movement and to stabilise the radial artery during the measurement. An arterial pulse wave sensor was placed on the skin directly over the radial artery at the point of the strongest pulse. The non-invasive acoustic sensor was adjusted to the highest relative signal strength, and the compliance measures were obtained during 30 s of blood pressure waveform collection. This device measures the decay in diastolic pressure in the large arteries and the decay in the reflective waves of the small arteries. Blood pressure and HR were measurements and also recorded at the same time. Three consecutive measures were collected and the average recorded. This non-invasive approach is repeatable and reliable both during long-term and short-term observations[22].

Heart rate variability

ECG recordings were taken supine for 20 min and recorded digitally using a biological amplifier (Bio Amp Model ML132, ADInstruments, Bella Vista, NSW, Australia) linked to a data acquisition system (Powerlab Model ML880, ADInstruments). ECG data were analysed offline by an assessor

Table 1. Composition of fatty acids in 1000 mg fish oil and placebo (sunola oil) capsules

Fatty acid	Fish oil (mg)	Placebo (mg)
14 : 0	30	–
14 : 1	2	–
15 : 0	10	–
15 : 1	1	–
16 : 0	204	38
16 : 1*trans*	5	–
16 : 1*n*-5	6	–
16 : 1*n*-7	36	1
16 : 1*n*-9	3	–
16 : 2*n*-4	1	–
16 : 3*n*-3	9	–
17 : 0	12	–
17 : 1	8	1
18 : 0	58	35
18 : 1*n*-7	21	–
18 : 1*n*-9	134	837
18 : 2*n*-6	14	63
18 : 3*n*-3	6	4
18 : 3*n*-6	2	–
18 : 4*n*-3	3	–
20 : 0	7	3
20 : 1*n*-11	12	3
20 : 1*n*-9	1	–
20 : 2*n*-6	3	–
20 : 3*n*-6	2	–
20 : 4*n*-3	2	–
20 : 4*n*-6	18	–
20 : 5*n*-3 (EPA)	56	–
22 : 0	2	–
22 : 1*n*-11	4	–
22 : 1*n*-9	3	–
22 : 4*n*-6	2	–
22 : 5*n*-3	10	–
22 : 5*n*-6	16	–
24 : 0	2	–
24 : 1	4	2
22 : 6*n*-3 (DHA)	262	–
Minor fatty acids	29	13

who was blinded to the treatments using the HRV Module 1.01 for Chart 5 (ADInstruments). Frequency domain parameters of HRV were derived using power spectrum analysis (fast Fourier transforms) with high-frequency power (defined as 0·15–0·40 Hz) and low-frequency power (defined as 0·04–0·15 Hz) expressed in normalised units adjusting for changes in total power. Poincare plots were used to determine the normal distribution of HRV. All ECG were recorded according to the standards of measurements, physiological interpretation and clinical use guidelines for the assessment of HRV[23].

Statistics

Statistical analysis was performed using SPSS for Windows 6.0 (SPSS, Chicago, IL, USA). Baseline characteristics between the treatment groups were compared using one-way ANOVA. A logarithmic scale was applied to the LF:HF data to ensure normal distribution for statistical analysis. The effects of the oil treatments on the dependent measures over time were analysed using random effects' mixed models, with oil treatment (dose of DHA-rich fish oil or control treatment) and time being the factors in the analysis, with time being the repeated measurement. For significant interactions, Bonferroni *post hoc* pairwise comparisons were performed to identify differences between means. Relationships between oil doses and changes in the dependent measures by week 12 were determined by linear regression. Changes were compared against baseline values to detect any regression to the mean. If the latter was found to be significant, a general linear model was used to assess treatment-related effects with baseline as a covariate. Statistical significance was set at $P<0·05$. All data are shown as mean (SEM).

Results

Study population

Seventy-five participants were recruited for the study. A subgroup of fifty-three participants agreed to undergo HRV assessment. Eight participants withdrew from the trial due to time constraints or other factors unrelated to the study. Out of these, four were in the group who underwent HRV assessment. One of the participants in the HRV assessment suffered an anxiety attack the day before testing at week 12 and did not complete this assessment. Additionally, data from two participants who underwent HRV assessment were excluded as statistical outliers (LF:HF for HRV was >3 standard deviations from the mean). Thus, at the end of the 12 week intervention, there were complete data on sixty-seven participants for LAC, blood pressure and HR and on forty-six participants for HRV.

Baseline (week 0) characteristics are shown for all participants in Table 2 and for the subgroup of participants who underwent HRV assessments in Table 3. No difference in any parameter was noted between treatment groups at baseline and there was no significant effect of sex. Baseline and week 12 measurements of LC *n*-3 PUFA content in erythrocytes have already been published elsewhere[21].

Table 2. Dose-related effects of fish oil supplementation for 12 weeks in all study participants
(Mean values with their standard errors)

	0 g/d (*n* 10 M/7 F)*				2 g/d (*n* 7 M/9 F)*				4 g/d (*n* 9 M/8 F)*				6 g/d (*n* 10 M/7 F)*			
	Baseline		Week 12		Baseline		Week 12		Baseline		Week 12		Baseline		Week 12	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Age (years)	52·6	2·5			53·4	2·2			54·0	2·1			54·0	1·6		
BMI (kg/m^2)	30·8	0·8	30·9	0·9	32·4	1·1	32·5	1·1	31·5	1·2	31·4	1·2	32·2	1·3	32·2	1·3
SBP (mmHg)	129·4	2·7	127·6	2·6	124	1·9	124·2	2·6	123·1	2·5	121·8	2·3	137·6	4·3	134·3	4·3
DBP (mmHg)	75·6	2·1	73·3	2·5	72·0	1·8	73·0	2·0	70·1	2·3	69·1	2·0	79·2	2·4	78·5	2·4
MAP (mmHg)	94·0	2·1	92·4	2·3	90·2	1·6	91·0	2·0	88·3	2·1	87·1	2·2	99·8	3·5	98·0	3·2
HR (bpm)	59·5	1·4	60·2	2·1	61·1	1·6	61·7	1·5	59·5	1·3	58·6	1·4	61·4	1·6	60·2	2·1
LAC (ml/mmHg × 10)†	17·1	1·0	16·5	0·86	17·6	0·7	17·8	0·66	15·3	1·1	15·8	1·41	14·6	0·78	16·7‡	0·92
SAC (ml/mmHg × 100)	8·8	0·97	9·1	0·9	8·9	0·81	8·2	0·75	7·67	0·72	8·8	0·9	7·5	0·89	7·0	0·8

M, male; F, female; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate; LAC, large artery compliance; SAC, small artery compliance.
*Volunteers (*n* 67) consumed 0, 2, 4 or 6 g/d of fish oil.
†There is a significant dose × time ($P<0·05$) effect of LAC.
‡Mean week 12 values significantly different from mean baseline value using Bonferroni *post hoc* pairwise comparisons ($P<0·05$).

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Table 3. Dose-related effects of fish oil supplementation for 12 weeks in the heart rate (HR) variability subgroup*
(Mean values with their standard errors)

	0 g/d (*n* 8 M/6 F)†				2 g/d (*n* 5 M/6 F)†				4 g/d (*n* 5 M/6 F)†				6 g/d (*n* 5 M/5 F)†			
	Baseline		Week 12		Baseline		Week 12		Baseline		Week 12		Baseline		Week 12	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Age (years)	51·4	2·9			52·2	2·7			53·9	2·9			54·3	2·1		
BMI (kg/m^2)	30·0	0·8	30·1	0·8	32·3	1·6	32·0	1·5	31·4	1·8	31·2	1·8	34·2	1·7	34·2	1·8
SBP (mmHg)	129·1	3·3	126	3·0	124·2	2·1	124·1	3·4	124·8	3·7	122	3·4	142·2	6·5	138·6	6·2
DBP (mmHg)	74·7	2·3	71·6	2·8	73·4	1·9	74·2	2·6	71·0	3·2	69·1	2·4	81·4	3·5	80·8	3·5
MAP (mmHg)	92·9	2·4	90·6	2·4	90·3	1·9	92·0	2·6	88·8	3·1	87·2	3·2	104·4	5·1	101·7	4·7
HR (bpm)	62·9	1·9	60·8	1·7	66·0	2·2	62·0	1·9	62·3	1·7	58·2	2·1	64·6	2·7	57	2·7
LAC (ml/mmHg x 10)	17·4	1·1	16·6	0·9	17·2	0·7	17·0	0·5	14·6	1·6	15·0	1·7	14·7	1·0	17·6‡	1·2
SAC (ml/mmHg x 100)	8·8	0·9	9·7	1·2	9·3	0·9	8·9	0·9	7·2	0·86	9·1	1·1	6·4	1·0	6·2	1·0
LF (nu)	74·7	3·1	71·6	4·1	71·8	3·2	72·6	3·6	70·5	3·6	62·3	5·4	79·1	1·9	70·4‡	2·8
HF (nu)	25·3	3·1	28·4	4·1	28·2	3·2	27·4	3·6	29·5	3·6	37·7	5·4	20·9	1·9	29·6‡	2·8
LF:HF (ratio)§	4·0	0·73	3·8	0·76	3·0	0·41	3·5	0·8	2·9	0·44	2·2‡	0·39	4·3	0·66	2·8‡	0·48

M, male; F, female; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; LAC, large artery compliance; SAC, small artery compliance; LF, low frequency; HF, high frequency.
*Forty-six out of the sixty-seven volunteers undertook assessments of HRV (see Methods).
† Participants consumed 0, 2, 4 or 6 g/d of fish oil.
‡ Mean week 12 values significantly different from mean baseline value using Bonferroni *post hoc* pairwise comparisons ($P<0·05$).
§ There is a significant dose × time ($P<0·05$) effect of LF:HF ratio.

Arterial compliance and blood pressure

There was a significant time × dose ($P=0·027$) effect for LAC, and *post hoc* tests revealed that, compared with placebo, the 6 g dose elicited a significant ($P<0·001$) improvement from weeks 0 to 12. Changes in LAC were correlated with dose of fish oil (r 0·34, $P=0·006$) but there was no relationship with small artery compliance. Changes in LAC were also correlated with changes in erythrocyte DHA content ($P=0·02$, r 0·29), but not with changes in erythrocyte EPA ($P=0·077$, r 0·22). Although there were no significant differences between the groups at baseline, regression analysis showed that the change in LAC correlated with baseline LAC (r −0·41, $P<0·001$), raising the possibility that the result may have been influenced by regression to the mean. However, analysis by a general linear model comparing treatment groups with placebo showed the following levels of significance for effects of treatment: 2 g/d, $P=0·408$; 4 g/d, $P=0·784$; 6 g/d, $P=0·004$. Hence, there was a highly significant treatment effect at the highest dose, as well as a significant correlation with dose, as noted above.

Heart rate

There was no significant effect in time ($P=0·74$) or dose ($P=0·70$) with HR. There was no correlation between change in HR and change in erythrocyte DHA ($P=0·34$, r −0·12) or EPA (r −0·022, $P=0·86$) content. However, changes in HR over 12 weeks correlated significantly with the corresponding changes in LAC (r −0·48, $P<0·001$).

Heart rate variability

The individual LF and HF components of HRV showed no dose × time effect or correlation with changes in erythrocyte DHA or EPA content or fish oil dose. However, there was a significant dose × time ($P=0·022$) effect for the LF:HF ratio; *post hoc* tests revealed significant differences from weeks 0 to 12 for the 4 g ($P=0·0049$) and 6 g ($P=0·0015$) doses *v.* placebo. The LF:HF ratio for HRV decreased with increasing dose of fish oil (r −0·34, $P=0·023$). There was no correlation of change in LF:HF ratio with change in erythrocyte DHA content (r 0·23, $P=0·13$), but there was a strong correlation with change in erythrocyte EPA content (r 0·47, $P<0·001$). Changes in the LF:HF ratio were inversely related to the corresponding changes in both LAC (r −0·31, $P=0·04$) and MAP (r 0·11, $P=0·024$).

Discussion

The present study demonstrates that dietary supplementation with DHA-rich fish oil over a 12-week period can produce dose-related improvements in both LAC and HRV. Even though these were secondary outcome measures in a broader-based study of dose-related cardiovascular benefits of DHA-rich fish oil[21], retrospective assessments indicate that there was 90 and 60 % power, respectively, to detect significant treatment effects in LAC and LF:HF ratio at $P=0·05$. Moreover, confidence in these outcomes is strengthened by the accompanying observations of significant dose relationships (linear regression) and inter-relationships (LAC changes

correlate with LF:HF ratio changes). Impaired arterial compliance and HRV are independent risk factors for CVD[1–3,24]; hence, the dose-related increases in both of these parameters suggest that increased intakes of DHA-rich fish oil are likely to be associated with dose-related reductions in CVD.

Raised HR *per se* is a risk factor for CVD[16]. A recent study utilising the HR-lowering drug ivabradine has found that reducing HR below 70 bpm reduced the incidence of CHD[25]. There is strong evidence that regular consumption of LC *n*-3 PUFA can also reduce HR. In a meta-analysis of thirty-two trials, Mozaffarian *et al.* [16] found that fish oil consumption for greater than 12 weeks reduced HR, particularly with groups that had a resting HR equal to or above 69 bpm. However, in the present study, we did not find a significant reduction in resting HR, perhaps because resting HR in the present study was below 69 bpm for each group. Nevertheless, the dose-related changes in LAC were significantly correlated with changes in HR.

The dose-related increase in LAC may have facilitated the improvement in HRV by increasing baroreflex sensitivity, a possibility that is supported by the observed correlations between changes in LAC and changes in both HR and LF:HF ratio. The responsiveness of stretch-sensitive afferent baroreceptors within the arterial wall would be facilitated by an increase in LAC, resulting in heightened baroreceptor sensitivity and afferent input leading to improved autonomic regulation of HRV. We also found that increasing HRV correlated with a decreasing MAP, again suggesting enhanced baroreflex activity.

The increase in LAC was related predominantly to DHA incorporation in erythrocytes, which is consistent with previous studies[26,27].On the other hand, the reduction in LF:HF ratio with increasing dose of fish oil, which indicates an increasing shift toward parasympathetic regulation, appeared to be mediated predominantly by EPA. EPA has been associated with a lower incidence of death from CHD and arrhythmias[28], and it is known that, compared with DHA, EPA is more readily incorporated into human atrial tissue[29]. In animal models, dietary fish oils have been shown to confer resistance to atrial fibrillation[30].The incorporation of EPA into atrial tissue is thought to be antiarrhythmic by virtue of EPA's ability to displace arachidonic acid, which is known to have pro-arrhythmic properties[31,32]. Thus, increased consumption of EPA and DHA may possibly improve HRV by both local and baroreflex modulation of sinoatrial function.

In conclusion, the observed relationships between fish oil dose and changes in LAC and LF:HF ratio suggest that regular fish oil supplementation can improve the regulation of HR, HRV and consequently blood pressure by increasing parasympathetic regulation of cardiac autonomic tone in a dose-dependent manner. These combined benefits may be expected to reduce CVD risk and provide further justification for increased intakes of fish oil.

Acknowledgements

The project was supported by an ARC Linkage Project number LP0561211 with Bartlett Grain Pty Ltd and Australian Pork Ltd. NuMega Ingredients kindly donated the fish oil and placebo supplements. We also acknowledge Amanda Jager, Keren Kneebone, Tahna Pettman and Erin Riley for their technical and administrative help with the project and John Petkov for his assistance with statistical analysis of the data. N. J. S. and C. M. M. contributed to data collection, data entry, data analysis and data interpretation. C. M. M. also contributed to recruitment and study design. A. M. C., J. D. B. and P. R. C. H. contributed to study design and conception, data interpretation, sourcing funding support and project management. D. A. S. contributed to the study conception and data interpretation. All authors contributed to manuscript preparation.

We declare that there are no conflicts of interest.

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