Docosahexaenoic acid-rich fish oil improves heart rate variability and heart rate responses to exercise in overweight adults

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Dietary fish oil supplementation and regular physical activity can improve outcomes in patients with established CVD. Exercise has been shown to improve heart rate variability (HRV), a predictor of cardiac death, but whether fish oil benefits HRV is controversial. Obese adults at risk of future coronary disease have impaired HRV and may benefit from these interventions. We evaluated the effect of DHA-rich tuna fish oil supplementation with and without regular exercise on HRV in sedentary, overweight adults with risk factors for coronary disease. In a randomised, double-blind, parallel comparison, sixty-five volunteers consumed 6 g fish oil/d (DHA 1·56 g/d, EPA 0·36 g/d) or sunflower-seed oil (placebo) for 12 weeks. Half of each oil group also undertook regular moderate physical activity (3 d/week for 45 min, at 75 % of age-predicted maximal heart rate (HR)). Resting HR and the HR response to submaximal exercise were measured at weeks 0, 6 and 12. In forty-six subjects, HRV was also assessed by power spectrum analysis of 20 min electrocardiogram recordings taken supine at baseline and 12 weeks. Fish oil supplementation improved HRV by increasing high-frequency power, representing parasympathetic activity, compared with placebo ($P=0\cdot01$; oil × time interaction). It also reduced HR at rest and during submaximal exercise ($P=0\cdot008$; oil × time interaction). There were no significant fish oil × exercise interactions. Dietary supplementation with DHA-rich fish oil reduced HR and modulated HRV in keeping with an improved parasympathetic–sympathetic balance in overweight adults with risk factors for future coronary disease.

Fish oil: Docosahexaenoic acid: Exercise: Coronary risk factors

Heart rate variability (HRV) is a non-invasive measure of cardiovascular risk which has been shown to predict cardiovascular death both in patients with established coronary disease[1–3] and in the general population[4,5]. A number of interventions that have been shown to reduce cardiovascular death rates also improve HRV, suggesting that they might improve survival by modulating the autonomic nervous system and reducing fatal cardiac arrhythmias.

Dietary fish consumption is associated with lower cardiovascular death rates[6] and dietary interventions to increase fish oil consumption reduce cardiovascular death without a reduction in myocardial infarction[7,8], implying an anti-arrhythmic effect. The mechanism for this is unclear but it has been suggested that this effect may be mediated through changes in autonomic function[9].

Fish or fish oil consumption has been shown to lower resting heart rate (HR)[10,11]. Some studies have also shown that fish oil improves HRV in a number of patient groups[12–15], while others have shown no effect[16–20]. Whether there is any effect of fish oil supplementation on HRV in an overweight and obese population, free of cardiac complications, has not been reported. This is of particular interest as the prevalence of overweight and obesity is increasing, and overweight and

obese adults are at risk of future CVD and have reduced HRV[21]. Additionally, regular aerobic exercise may also lower resting HR and improve HRV[22–24]. Whether aerobic exercise can accentuate the benefits of fish oil is unknown. We examined the effects of regular aerobic exercise and supplementation with DHA-rich fish oil on resting HR, HRV and HR responses to submaximal exercise in overweight and obese subjects with risk factors for coronary disease. DHA purportedly offers greater cardiovascular benefits than other *n*-3 fatty acids[25] but its effects on HRV are unclear.

Methods

Protocol outline

Sedentary, overweight (BMI $> 25\,\text{kg/m}^2$) adults aged 25–65 years with additional risk factors for CVD (mild hypertension (systolic blood pressure (BP) 140–160 mmHg or diastolic BP 90–100 mmHg), elevated plasma TAG ($> 1\cdot6$ mmol/l) or elevated total cholesterol ($> 5\cdot5$ mmol/l)) were recruited as part of a larger study investigating the cardiovascular and metabolic effects of DHA-rich fish oil and regular moderate aerobic exercise. Seventy-five subjects began the study; of these, fifty

subjects also volunteered to undergo HRV assessment. The subject recruitment and treatment allocation details are described elsewhere[26]. Briefly, subjects were recruited over a 2-year period. Individuals were first required to complete a diet and lifestyle questionnaire to determine initial suitability. Exclusion criteria included: diabetes, liver disease, or CVD; BP- or lipid-lowering medication; exercise more than once per week for the purpose of improving health; consumption of fish-oil capsules or more than one fatty fish meal per week; pregnancy or lactation; or currently on a weight-reduction diet. A total of 315 individuals were invited to attend a screening appointment where a fasting blood sample, height, weight and resting BP were obtained. Individuals who met the eligibility requirements were asked to complete a supervised graded exercise test to determine suitability for exercise training. Seventy-five subjects were randomised in a double-blind 2×2 factorial design to a 12-week intervention where they consumed 6 g/d of either DHA-rich fish oil, providing 0·36 g EPA/d and 1·56 g DHA/d (Hi-DHA®; Nu-Mega Ingredients Pty Ltd, Brisbane, Australia), or sunflower-seed oil. Subjects in each group were also randomised to undertake either three sessions of aerobic exercise per week for 45 min at a HR which corresponded to 75 % of their age-predicted maximum $(208 - (0.7 \times \text{age}))$[27], or no formal exercise. The four groups were therefore identified as: fish oil; fish oil and exercise; sunflower-seed oil; sunflower-seed oil and exercise. All subjects were invited to participate in the HRV sub-study. For this HRV sub-study, subjects needed to be in sinus rhythm.

HR was assessed at baseline and after 6 and 12 weeks of intervention; HRV was assessed at baseline and 12 weeks only. All subjects were instructed to maintain their normal diet and physical activity patterns during the study, apart from undertaking the exercise programme and/or supplementation as required.

Ethics approval was obtained from the Human Research Ethics Committees of both the University of Adelaide and the University of South Australia and written informed consent was obtained from all subjects before participation.

Heart rate and exercise testing

Resting HR was recorded supine using the HDI/PulseWave™ CR-2000 Cardiovascular Profiler (Hypertension Diagnostics Inc., Eagan, MN, USA) as the mean HR over 10 min following a 10 min rest period. The HR response to exercise was measured during a graded submaximal exercise test using a Polar Accurex Plus Heart Rate monitor (Sports Tester; Polar Electro Oy, Kempele, Finland). During the graded exercise test subjects stood for 2 min before beginning to walk at a self-selected moderate walking speed at 0 % incline on an electronic treadmill (model Q65; Quinton Instruments, Seattle, WA, USA). After 10 min, the incline was increased to 5 % grade for 5 min and then 10 % grade for an additional 5 min. During a 2 min rest period at the end of each workload, subjects were seated. The average HR during the last 1 min of each workload was used for analysis.

Heart rate variability

Electrocardiogram (ECG) recordings were taken supine for 20 min at baseline and following 12 weeks of intervention.

The ECG were recorded digitally using a biological amplifier (Bio Apm model ML132; ADInstruments, Bella Vista, NSW, Australia) and data acquisition system (Powerlab model ML880; ADInstruments). ECG data were analysed off-line by two independent blinded investigators using the HRV Module 1·01 for Chart 5 (ADInstruments).

Frequency domain parameters of HRV were derived using power spectrum analysis with high-frequency power (HFP; defined as 0·15–0·40 Hz) and low-frequency power (LFP; defined as 0·04–0·15 Hz) expressed in normalised units adjusting for changes in total power (which is related to HR). Normalised frequency domain parameters were chosen due to the short-term ECG recordings to minimise the effect of changes in HR. These parameters correlate well with time domain parameters from 24 h ECG recordings[28]. HFP reflects parasympathetic activity and LFP reflects a combination of sympathetic and parasympathetic activity. The ratio LFP:HFP is thought to represent a measure of autonomic balance.

Statistical analysis

Statistical analysis was performed using Statistica for Windows (version 5.1; StatSoft Inc., Tulsa, OK, USA). Baseline characteristics between the treatment groups were compared using one-way ANOVA for resting HR, and two-way ANOVA for exercise HR (treatment × workload). The effect of DHA-rich fish oil supplementation and exercise on HRV and their interactions was analysed using three-factor repeated-measures ANOVA with oil treatment (fish or sunflower-seed oil) and exercise treatment (exercise training or non-exercise) being the between-group factors and time being the within-subject repeated measurement. The effects of treatment on HR at rest and during incremental exercise were assessed by four-factor ANOVA (oil treatment, exercise treatment, exercise condition (i.e. resting or HR at end of each workload during incremental exercise) and time). P values of < 0.05 were considered statistically significant. Results are presented as mean values with their standard errors.

Results

Subject characteristics

The characteristics of the subject group in the larger study, as well as their group allocation and attrition patterns, have been described previously[26]. From the seventy-five subjects who entered the larger study, data for sixty-five subjects were available for analysis of changes in resting HR. Data for HR response to submaximal exercise were available on fifty-nine subjects. Paired ECG recordings suitable for HRV analysis were obtained from forty-six subjects after four failed to complete the study due to difficulties with the prescribed exercise. Neither subgroup differed in baseline characteristics from the larger cohort. All forty-six subjects had a BMI $> 25 \text{ kg/m}^2$; thirty-four had elevated cholesterol, thirty-one had elevated TAG and twenty-two had mild hypertension. Baseline characteristics for the forty-six subjects who completed HRV assessments at weeks 0 and 12 are shown in Table 1. There were no significant differences between treatment groups for any of these characteristics.

Table 1. Baseline characteristics for participants who underwent heart rate variability assessment

(Mean values and ranges)

Treatment groups...	FO (n 13)		FOX (n 10)		SO (n 14)		SOX (n 9)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Age (years)	52	40–63	48	37–52	51	31–61	50	29–60
Sex								
Male (n)	4		4		6		3	
Female (n)	9		6		8		6	
BMI (kg/m^2)	35·3	27·5–47·8	32·6	27·8–45·3	34·3	27·7–41·1	32·7	25·7–38·8
Cholesterol (mmol/l)	5·8	4·7–7·2	5·8	2·3–7·7	5·9	4·3–7·4	5·8	4·4–7·9
TAG (mmol/l)	1·5	1·0–2·4	2·1	0·9–8·0	1·7	0·9–4·6	2·1	0·8–3·4
Systolic BP (mmHg)	127	105–148	130	111–154	126	107–148	131	114–147
Diastolic BP (mmHg)	72	56–89	77	67–96	72	60–83	75	66–93

FO, fish oil; FOX, fish oil and exercise; SO, sunflower-seed oil; SOX, sunflower-seed oil and exercise; BP, blood pressure.

More than 85 % of subjects allocated to an exercise group completed the required number of training sessions. All subjects consumed the required number of capsules, as determined by capsule counts. The changes in blood lipids and erythrocyte fatty acid composition associated with fish oil supplementation have been described elsewhere[26]. There was no change in weight or BP over the 12 weeks in any group.

Heart rate

There was no difference in resting HR ($P > 0.96$) or HR at each workload during submaximal exercise ($P > 0.94$) between groups at baseline (week 0). HR increased progressively from rest with increasing workload during submaximal exercise ($P < 0.001$). There was a significant oil treatment × time interaction ($P = 0.008$) as a result of a greater reduction in resting and exercise HR in the subjects consuming the fish oil supplements compared with those consuming the sunflower-seed oil supplements (Table 2). There were no significant oil × exercise ($P = 0.34$) or exercise × time ($P = 0.19$) interactions.

Heart rate variability

There were no differences in the baseline frequency domain measurements of HRV between the treatment groups ($P > 0.21$).

There was a significant oil × time interaction ($P = 0.01$) for HFP due to an increase in HFP in subjects consuming the fish oil supplements compared with those consuming sunflower-seed oil (Fig. 1 (a); Table 3). There were no significant exercise × time ($P = 0.89$) or oil × exercise ($P = 0.90$) interactions.

There were no significant effects of either exercise or fish oil supplementation on the LFP:HFP ratio, although the interaction between oil treatment and time approached significance ($P = 0.06$; Table 3).

There was a significant exercise × time interaction ($P = 0.01$) for LFP due to a reduction in LFP in subjects who were not in the exercise treatment groups compared with those who were exercising (Fig. 1 (b); Table 3). There were no significant oil × time ($P = 0.46$) or oil × exercise ($P = 0.93$) interactions.

Discussion

A positive association between n-3 PUFA intake and HRV has been reported in adults at high risk for cardiovascular events, including patients who are post-myocardial infarction[29], presenting for angiography[30], have diabetes[31] or who have chronic renal failure[12]. Associations between n-3 fatty acids and HRV have also been demonstrated in the elderly[15] and in healthy men[13,32]. Furthermore, intervention studies have shown that dietary supplementation with fish oil can improve HRV in patients following myocardial infarction[14,33], patients with chronic renal failure[12] and in elderly nursing-home patients[15]. In contrast, other studies have shown no improvement in HRV with fish oil supplementation following myocardial infarction[19], in dialysis patients[20] and in healthy subjects[17,18]. In a healthy population, Christensen et al.[13] reported a dose–response relationship between fish oil supplement intake and HRV in men with low baseline HRV, but a similar dose–response relationship was not observed in women. These conflicting results may be explained by differences in populations, fish oil doses and the HRV parameters studied (for example, whether the results were adjusted for changes in HR).

In the present study we demonstrated an effect of dietary fish oil supplementation on HRV in obese sedentary adults at risk of future coronary disease. We observed an increase in HFP consistent with enhanced parasympathetic modulation of HR without a significant effect on LFP, which is thought to reflect sympathetic activity. This pattern of improved parasympathetic modulation following fish oil supplementation has been demonstrated consistently across a number of populations with impaired HRV[12–15], but has not previously been shown in an overweight or obese population.

Both regular fish consumption[10] and supplementation with fish oil have been shown to lower resting HR[11]. In a large population-based trial, Mozaffarian et al.[34] evaluated the association between HR and usual dietary intake of EPA + DHA. They observed a steep decline in HR with doses up to 0·3 g/d, followed by a gradual reduction with doses increasing to 1·5 g/d. The highest fish intake, which provided EPA + DHA at about 1·07 (SEM 0·44) g/d, was associated with a 3·2 beats per min reduction in resting HR compared with no fish intake. This difference is comparable with the approximate 2·3 beats per min reduction in resting HR observed in the

Table 2. Heart rate (HR) at rest and during exercise
(Mean values with their standard errors)

	Oil treatments*				Exercise treatments			
	Fish oil		Sunflower-seed oil		Exercise		No exercise	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Resting HR (bpm) (about 0·9 MET)								
Week 0	62·2	1·3	61·5	1·4	62·0	1·6	61·6	1·2
Week 6	60·3	1·4	61·6	1·5	60·1	1·7	61·7	1·3
Week 12	59·9	1·4	61·5	1·3	59·7	1·6	61·5	1·0
Change week 12 − week 0	−2·3	1·0	0·0	1·0	−2·3	1·0	−0·1	1·0
Workload 1 HR (bpm) (about 2·4 MET)								
Week 0	98·2	2·6	95·0	1·8	96·8	2·1	96·4	2·4
Week 6	93·6	2·0	93·7	1·9	94·2	2·0	93·1	1·9
Week 12	89·0	1·4	91·1	1·6	89·1	1·3	90·9	1·6
Change week 12 − week 0	−9·1	1·8	−3·9	1·3	−7·7	1·6	−5·5	1·6
Workload 2 HR (bpm) (about 3·3 MET)								
Week 0	109·1	2·6	107·8	2·1	109·2	2·4	107·7	2·3
Week 6	103·2	1·8	105·8	1·9	105·0	1·8	104·0	1·9
Week 12	100·3	1·7	104·9	1·8	101·5	1·7	103·6	1·9
Change week 12 − week 0	−8·7	1·7	−3·0	1·3	−7·7	1·7	−4·1	1·4
Workload 3 HR (bpm) (about 4·5 MET)								
Week 0	129·2	2·9	128·6	2·9	129·2	2·9	128·6	2·8
Week 6	120·9	2·1	125·3	2·5	122·5	2·2	123·6	2·5
Week 12	118·9	2·3	124·2	2·4	119·8	2·3	123·1	2·5
Change week 12 − week 0	−10·4	1·6	−4·3	1·4	−9·4	1·6	−5·4	1·5

bpm, Beats per min; MET, metabolic equivalents.
* Significant oil × time interaction ($P = 0.008$).
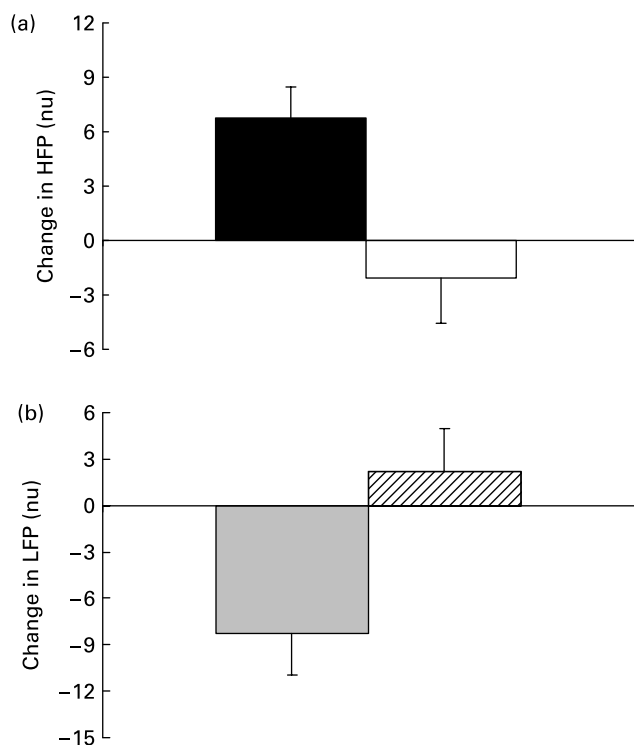
Fig. 1. Changes in indices of heart rate variability following 12 weeks of intervention. (a) Effect of fish oil (■) and sunflower-seed oil (□) on high-frequency power (HFP). There is an oil × time interaction ($P = 0.01$). (b) Effect of exercise (▧) or no formal exercise (▨) on low-frequency power (LFP). There is an exercise × time interaction ($P = 0.01$). nu, Normalised units. Values are means, with their standard errors represented by vertical bars.

subjects supplemented with fish oil (fish oil, and fish oil and exercise) in the present study.

A number of studies have shown a reduction in resting HR following regular aerobic exercise training[22–24,35–37], although others have reported no effect[38–42]. Similar inconsistencies exist for effects of exercise on HRV[22,24]. These differences may be due to differences in measurement of HRV, the time, duration and intensity of the exercise intervention and the population studied. The lack of effect of exercise on resting or exercise HR and HRV in the present study may reflect the nature of the exercise regimen, which was chosen as a realistic lifestyle modification which was sufficiently modest in terms of frequency, intensity and duration that it should be sustainable for most individuals in this population. The only effect of exercise was that it attenuated the reduction in LFP that was seen in subjects who did not undertake exercise. LFP reflects components of both sympathetic and parasympathetic modulation of HRV[43]. Exercise programmes similar to that used in the present study have been shown to improve baroreceptor sensitivity and increase sympathetic modulation of HRV (i.e. increase LFP)[43]. This effect of exercise on LFP may explain why LFP was maintained relative to the reduction that was seen in subjects who did not exercise.

Supplementation with DHA-rich fish oil significantly attenuated the HR response to submaximal exercise, an effect that was independent of exercise training. This effect has also been observed in patients with stable coronary artery disease[44], although with substantially greater doses of n-3 fatty acids (5·4 g/d). Two short-term studies found no effect of fish oil on peak HR during exercise[33,45]. The lower HR at submaximal workloads, without any effect on peak HR, indicates that fish oil improves cardiac efficiency without negatively impacting on the ability to maximise HR responses. O'Keefe et al.[33]

Table 3. Heart rate variability parameters
(Mean values with their standard errors)

	Oil treatments				Exercise treatments			
	Fish oil (n 23)		Sunflower-seed oil (n 23)		Exercise (n 19)		No exercise (n 27)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
HFP (nu)*								
Week 0	37·61	4·04	37·54	2·56	37·89	3·35	37·36	3·32
Week 12	44·37	4·25	35·48	2·80	40·18	3·10	39·74	3·91
LFP (nu)†								
Week 0	56·03	3·98	58·00	2·63	53·32	3·01	59·62	3·38
Week 12	50·66	4·24	55·55	3·76	55·55	3·21	51·38	4·27
LFP:HFP ratio								
Week 0	2·63	0·60	1·70	0·19	1·61	0·27	2·55	0·50
Week 12	2·00	0·41	2·29	0·59	1·85	0·28	2·35	0·58

HFP, high-frequency power; nu, normalised units; LFP, low-frequency power.
* Significant oil × time interaction ($P=0.01$).
† Significant exercise × time interaction ($P=0.01$).

reported a reduction in resting HR and an improved HR recovery after exercise in cardiac patients supplemented with 0·81 g n-3 fatty acids/d. This is particularly important as HR recovery after exercise is an independent predictor of mortality[46].

Dietary fish oil may modulate HR at rest and during exercise by a number of mechanisms. Dietary n-3 fatty acids are incorporated into cardiomyocyte membranes and this may modulate ion channel function in the pacemaker cells of the sinus node[47]. n-3 Fatty acids also have complex effects on lipids, BP and circulatory function that could result in improved cardiac efficiency, which would be reflected in a reduced HR for a given workload[48,49]. By improving arterial elasticity, fish oil may also increase the sensitivity of baroreflex stretch receptors and consequently arterial baroreflex gain, which is mediated by cardiovagal modulation of HR. Thus the increased baroreflex sensitivity is reflected in increased HRV.

The relative influence of specific n-3 fatty acids on HR and HRV remains controversial, as many studies, including the present study, have used combinations of EPA and DHA and in differing proportions. There are only limited clinical data on the cardiovascular effects of purified EPA and DHA and it has been suggested that they may have differential effects in different populations[25]. Brouwer et al.[32] showed a strong association between DHA but not EPA and HRV. Similarly, Christensen et al.[13] reported that the EPA content in granulocytes correlated with mean 24 h R-R intervals (duration of ventricular cardiac cycle), while DHA content was associated with all six indexes of HRV. Other investigators have shown that DHA but not EPA improves BP, resting HR and endothelial function[50–52]. It should be noted that DHA is the predominant long-chain n-3 fatty acid in fish, the consumption of which is associated with improvements in HR[10,34] and HRV[9], and while the fish oil supplements used in the present study contained both EPA and DHA, the supplement was a DHA-rich oil.

Conclusion

Dietary fish oil supplementation, which delivered 1·56 g DHA/d and 0·36 g EPA/d, increased parasympathetic measures of HRV and lowered the HR response to exercise in subjects with risk factors for coronary artery disease. This provides further evidence supporting the use of dietary fish oil as a supplement, not only for patients with established coronary disease, but also for those at risk of future CVD.

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