**Cardiac Autonomic Changes Associated With Fish Oil vs Soy Oil Supplementation in the Elderly***

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**Introduction:** Omega-3 fatty acid levels are associated with decreased risk for sudden death; however, the protective cardiovascular mechanisms of omega-3 are poorly understood. This study addresses the heart rate variability (HRV) changes in a cohort of elderly subjects randomized to receive either a daily high dose of marine-derived omega-3 fatty acids (fish oil) or a lower daily dose of a plant-derived omega-3 fatty acid (α-linolenic acid) in soy oil.

**Methods:** A total of 58 elderly nursing home residents were randomized to receive 2 g/d of fish oil capsules vs 2 g/d of soy oil capsules, and were subsequently followed up every other day for a period of 6 months with 6-min measurements of HRV while resting supine. An initial control period of 2 months without supplementation was allowed to establish an HRV baseline for each participant.

**Results:** The average time- and frequency-domain parameters of HRV increased significantly during the supplementation period in both the fish oil and soy oil groups. In the regression model after adjusting for age and mean heart rate, supplementation with fish oil was associated with a significant increase in the high- and low-frequency components, and SD of normal RR intervals (SDNN), whereas only SDNN increased significantly in the soy oil group.

**Conclusions:** Supplementation with 2 g/d of fish oil was well tolerated and was associated with a significant increase in HRV. Supplementation with 2 g/d of soy oil was associated with a lesser but significant increase in HRV.

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**Key words:** autonomic; elderly; fish oil; heart rate variability; soy oil

**Abbreviations:** ALA = α-linolenic acid; CI = confidence interval; HF = high frequency; HRV = heart rate variability; LF = low frequency; p-NN50 = percentage of successive normal RR intervals differing by > 50 ms; r-MSSD = root square root of the mean of the sum of the squares of differences between adjacent intervals; SDNN = SD of normal RR intervals

CARDIOVASCULAR DISEASE is one of the leading causes of mortality in industrialized nations, with approximately 60% of all cardiovascular deaths occurring in the form of sudden cardiac death.1 Omega-3 polyunsaturated fatty acids appear to be an effective form of primary prevention by reducing rates of sudden death in healthy male subjects with fish consumption of at least one meal of fish per week.2 Further, there is a dose-response relation with lower risk of sudden death in subjects with higher cell membrane concentrations of omega-3 fatty acids.3 In patients with underlying coronary artery disease, supplementation with omega-3 fatty acids is also associated with lower rates of cardiovascular mortal-
ity by reducing fatal myocardial infarction and sudden death rates. The reduction in mortality has been shown to be significant after only 3 months of omega-3 fatty acid supplementation and is independent of myocardial infarction rates; these findings suggest that omega-3 fatty acids function primarily as antiarrhythmic agents. One of the proposed mechanisms by which omega-3 fatty acids may decrease the risk of arrhythmia is by increasing heart rate variability (HRV), a measure of cardiac autonomic regulation. However, previous studies have not described the longitudinal effects of omega-3 fatty acids on HRV, and the HRV changes associated with nonmarine sources of omega-3 fatty acids are unknown. In this prospective study, we hypothesized that supplementation with either a daily high dose of marine-derived omega-3 fatty acids (fish oil) or a lower daily dose of a plant-derived omega-3 fatty acid (α-linolenic acid [ALA]) in soy oil can increase HRV in elderly subjects. The results presented here are part of a series of studies aimed at understanding and preventing the HRV effects of particulate air pollution on elderly populations.

**Materials and Methods**

**Study Population**

We recruited nursing home residents who signed a consent form and were able to undergo HRV measurements in the supine position, and excluded participants with the following: age < 60 years, cardiac pacemaker, cardiac arrhythmias other than isolated premature ventricular or atrial contractions, allergies to omega-3 fatty acids or fish, receiving oral anticoagulants other than aspirin, and bleeding diathesis. Patients were followed up with every-other-day measurements of HRV from September 27, 2001, to April 5, 2002. The study was approved by the Institutional Research Board of the National Institute of Public Health in Mexico.

**HRV Analysis**

Participants were scheduled to undergo an HRV recording every other day between 8 AM and 1 PM using an LPPac Q, Predictor 3.0 (Arrhythmia Research Technology; Houston, TX), which meets standards from the European Society of Cardiology and the North American Society of Pacing and Electrophysiology for the measurement of HRV. Participants were instructed to rest in a supine position for 5 min before starting the study, and each recording lasted for 6 min while resting in the supine position. Identification of a QRS template was done by an automated algorithm that identifies the wave form most representative of the patient’s dominant rhythm. This template was compared with subsequent R waves utilizing a correlation coefficient of 0.75 as previously described in the study by Liao et al. Any test having > 15% of abnormal QRS processes was discarded. The HRV editing was blinded as to the supplementation group assigned to each participant. We estimated the power spectral density utilizing a fast Fourier transformation and a Hanning window; with a smoothing weight of 10. Three frequencies were obtained from the power spectral density: (1) a high-frequency (HF) component (0.15 to 0.40 Hz), (2) a low-frequency (LF) component (0.04 to 0.15 Hz), and (3) a very-low-frequency component (0.033 to 0.04 Hz). We also obtained the following time-domain parameters: (1) SD of normal RR intervals (SDNN), percentage of successive normal RR intervals differing by > 50 ms (p-NN50), and (3) square root of the mean of the sum of the squares of differences between adjacent intervals (r-MSSD). All participants answered a questionnaire that included demographic information, smoking habits, symptom diary, and a validated food frequency questionnaire at the beginning of the study.

**Fatty Acid Supplementation**

Participants were randomized in a double-blind fashion to either fish oil or soy oil at study entry, and were subsequently followed up with every-other-day measurement of HRV for 2 months prior to any supplementation. The reason for the pre-supplementation phase was to establish a control period in which the HRV baseline for each participant could be determined. Patients in the omega-3 fatty acid group received 2 g/d in divided doses. Each 1-g capsule contained 53.2% of omega-3 fatty acids (docosahexaenoic acid and eicosapentaenoic acid). The soy oil group capsules were identical and were composed of 1 g of soy oil, which contained 6.78% of ALA, 16.3% of saturated fat, and 52.7% of linoleic acid (omega-6). Compliance was assessed by providing each capsule under direct supervision, and by pill count.

**Statistical Analysis**

Comparison of variables between the two groups was tested by a nonpaired t test and a two-sample Wilcoxon rank-sum (Mann Whitney). The χ² or Fisher exact tests were used for discrete variables and frequencies. We evaluated the effect of the omega-3 fatty acids over time using a random-effects regression model for repeated measures, which included an interaction term between the omega-3 fatty acids supplemented group and the time after the supplementation started (to test for different trends between the two supplements) and were also adjusted by age and heart rate. Since HRV variables have a skewed distribution, they were log-transformed. Gender, hypertension, and diabetes were not significant confounders and were not included in the model. This model allowed us to test whether the HRV associated with the fish oil or soy oil supplementation was different from the HRV baseline obtained during the 2-month control period in which no supplementation was given, and whether the time to achieving any such change in HRV differed between the supplements. In this type of analysis, every subject is his/her own control; p < 0.05 was considered significant. All statistical analyses were conducted using software (Stata 7.0; StataCorp; College Station, TX).

**Results**

Of a total of 58 patients, 52 were followed up for the complete study period, and 6 patients were unavailable for follow-up for the following reasons: transfer to another nursing home (n = 2), refusal to continue participating (n = 3), and death from complications related to an underlying malignancy after...
LF components, and SDNN when comparing HRV with a significant increase in the total HRV, HF, and rate, supplementation with fish oil was associated with a significant increase in the total HRV, HF, and LF domain parameters increased significantly during the supplementation period. In the soy oil group, except for the LF component, all HRV parameters also were significantly increased during the supplementation period. After adjusting for age and mean heart rate, supplementation with fish oil was associated with a significant increase in total HRV, HF, and LF components, and SDNN when comparing HRV measurements in the presupplementation and supplementation periods: 0.23 (95% confidence interval [CI], 0.10 to 0.35), 0.24 (95% CI, 0.10 to 0.38), 0.30 (95% CI, 0.16 to 0.43), and 20.17 (95% CI, 8.65 to 31.69), respectively. In contrast, the soy oil group was associated with a significant increased in SDNN (13.17; 95% CI, 1.72 to 24.61) and a marginal increase in the LF and HF components (Table 3).

Figure 1 show the presupplementation HRV (total power of the frequency domain) in the fish oil and soy oil groups and the changes in HRV during the supplementation phase. In the fish oil group, there was a significant increase in HRV during the first quartile of the supplementation period in contrast to the soy oil group, which showed a significant HRV increase until the third quartile of the supplementation period.

The compliance with fish oil and soy oil was 92.5% and 93.8%, respectively. The average consumption of dietary omega-3 fatty acids for the entire study population was poor, with only 9% of participants eating fish other than tuna, and 26% eating tuna once per week; fish consumption was similar in the fish oil and soy oil groups (data not shown). No significant clinical adverse effects were reported. Forty-one percent of patients in the fish oil group reported belching vs 16% in the soy oil group (p = 0.04), 12.5% of patients in the fish oil group vs 8% in the soy oil group reported having nausea, and there were no reports of bleeding or skin bruising.

**Discussion**

After adjusting for age and heart rate, supplementation with fish oil was associated with a significant increase in total HRV, HF and LF domain components, and SDNN. In participants randomized to soy oil, there was a marginally significant increase in the HF and LF components, and a significant increase in

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### Table 1—Demographic Characteristics, Diagnosis, and Medications of the Study Population in the Fish Oil and Soy Oil Supplementation Groups*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Fish Oil</th>
<th>Soy Oil</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td>26</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Female gender, %</td>
<td>54</td>
<td>50</td>
<td>0.78</td>
</tr>
<tr>
<td>Age (range), yr</td>
<td>76 (60–96)</td>
<td>77 (60–89)</td>
<td>0.58†</td>
</tr>
<tr>
<td>Current smoking, %</td>
<td>12</td>
<td>12</td>
<td>1.00</td>
</tr>
<tr>
<td>COPD</td>
<td>3</td>
<td>2</td>
<td>0.64</td>
</tr>
<tr>
<td>Diabetes</td>
<td>6</td>
<td>3</td>
<td>0.27</td>
</tr>
<tr>
<td>Hypertension†</td>
<td>10</td>
<td>8</td>
<td>0.56</td>
</tr>
<tr>
<td>Heart failure</td>
<td>4</td>
<td>0</td>
<td>0.04</td>
</tr>
<tr>
<td>β-blockers</td>
<td>2</td>
<td>1</td>
<td>0.55</td>
</tr>
<tr>
<td>Angiotensin-converting enzyme inhibitors</td>
<td>3</td>
<td>2</td>
<td>0.64</td>
</tr>
<tr>
<td>Diuretics</td>
<td>3</td>
<td>0</td>
<td>0.07</td>
</tr>
<tr>
<td>Calcium-channel blockers</td>
<td>2</td>
<td>3</td>
<td>0.64</td>
</tr>
</tbody>
</table>

*Data are presented as No. unless otherwise indicated.
†Two patients were labeled as hypertensives, but were not receiving any BP medications during the study.
‡log10 (ms²)/100,000.
SDNN. To our knowledge, this is the first study to show the HRV response in a prospective randomized study to different sources of omega-3 fatty acids.

Our results also show that a short supplementation time of 2 g/d of fish oil leads to a significant increase in HRV. This contradicts previous assertions that a prolonged time of omega-3 fatty acid intake is required to change cardiac autonomic function. In contrast, participants in the soy oil group required a longer time of supplementation to achieve a significant increase in HRV.

Reduced HRV predicts mortality and arrhythmic complications in patients after myocardial infarction and in apparently healthy middle-age and elderly subjects. Therefore, increasing HRV would seem an effective form of secondary and primary prevention to reduce cardiovascular mortality. In healthy subjects and in patients with coronary artery disease, there is a positive correlation between the baseline cell membrane concentrations of omega-3 fatty acid and the degree of HRV. Further, supplementation with omega-3 fatty acids leads to a dose-dependent increase in HRV. Other antiarrhythmic mechanisms of omega-3 fatty acids have also been described, including the capacity to stabilize the electrical activity of cardiac myocytes by modulating sarcolemmal ion channels and voltage-dependent sodium channels and the capacity to reduce myo-

### Table 3—Effect of the Fish Oil and Soy Oil Supplementation on the Frequency- and Time-Domain Parameters of HRV

<table>
<thead>
<tr>
<th>Variables</th>
<th>Fish Oil*</th>
<th>p Value</th>
<th>Soy Oil*</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>β (95% CI)</td>
<td>β (95% CI)</td>
<td>p Value</td>
<td>β (95% CI)</td>
<td>p Value</td>
</tr>
<tr>
<td>Total variability</td>
<td>0.23 (0.10–0.35)</td>
<td>0.001</td>
<td>0.08 (−0.03–0.20)</td>
<td>0.1</td>
</tr>
<tr>
<td>HF†</td>
<td>0.24 (0.10–0.38)</td>
<td>0.001</td>
<td>0.13 (−0.0023–0.28)</td>
<td>0.057</td>
</tr>
<tr>
<td>LF‡</td>
<td>0.30 (0.16–0.43)</td>
<td>0.001</td>
<td>0.12 (−0.009–0.26)</td>
<td>0.06</td>
</tr>
<tr>
<td>SDNN†</td>
<td>20.17 (8.65–31.60)</td>
<td>0.001</td>
<td>13.17 (1.72–24.61)</td>
<td>0.02</td>
</tr>
<tr>
<td>r-MSSD</td>
<td>7.49 (−4.44–19.43)</td>
<td>0.2</td>
<td>6.85 (−5.0–18.7)</td>
<td>0.2</td>
</tr>
<tr>
<td>p-NN50</td>
<td>0.77 (−1.6–3.22)</td>
<td>0.5</td>
<td>0.69 (−1.73–3.13)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*Adjusted for the age and mean heart rate, and interaction term between the omega-3 supplemented group and the time after the supplementation started.
†log10 (ms²)/100,000.
‡log10 (ms²).
cardiac infarct size in animal models of ischemia and reperfusion. Christensen et al failed to show significant correlations between the baseline membrane levels of ALA in platelets and granulocytes with 24-h indexes of HRV; however, this study only correlated baseline values without any dietary intervention. ALA can be desaturated and elongated into docosahexaenoic acid and eicosapentaenoic acid, yielding small increases of omega-3 fatty acids. It is therefore possible that the concentration of ALA in soy oil increased the levels of omega-3 fatty acids sufficiently to produce a lesser increase in HRV. The cardiac autonomic changes associated with soy oil supplementation, whether as an alternate source of omega-3 fatty acids and/or by a direct effect of ALA, provide a theoretical framework for the beneficial cardiovascular effects of ALA in Mediterranean diets.

Therefore, a diet supplemented with omega-3 fatty acids derived from either soy oil or fish oil in conjunction with other factors known to increase HRV such as exercise, weight loss, stress reduction, and restoration of normal sleep could be part of an effective way to improve cardiac autonomic function.

Several limitations must be considered when interpreting results from this study. The population was limited to subjects > 60 years old, and therefore these results may not be applicable to younger age groups. Also, this study was conducted in a group of nursing home residents who do not consume fish or other sources of omega-3 fatty acids regularly in their diets; therefore, these results may not be extrapolated to populations with higher fish intake. Because of the small number of participants in each group, we were unable to perform subgroup analysis by underlying medical diagnoses or type of medications used. Measurements of HRV were limited to 6-min recordings while resting in the supine position; therefore, we are unable to estimate the effect of fish oil or soy oil supplementation on very low frequencies of HRV. Although we did not measure omega-3 fatty acid levels in the participants, compliance was determined by direct observation and pill count, and was > 90% for both fish oil and soy oil groups.

Even though our study did not have a control group, the effect of fish oil and soy oil supplementation on each participant's HRV was estimated in reference to their baseline HRV during the 2-month presupplementation phase; therefore, each subject was his/her own control. The supplementation phase was limited to 11 weeks, and thus we are unable to make inferences on more long-term effects of omega-3 fatty acids.

To our knowledge, this study shows for the first time that soy oil supplementation (either through a direct effect of ALA or by increasing the levels of omega-3 fatty acids) can have antiarrhythmic properties by increasing HRV. Further research on the HRV effects of higher ALA doses needs to be explored; furthermore, whether the cardiac autonomic effects of ALA on HRV depend on its elongation into omega-3 fatty acids also needs to be determined.

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