Omega-3 Fatty Acid Supplementation Augments Sympathetic Nerve Activity Responses to Physiological Stressors in Humans

Kevin D. Monahan, Thad E. Wilson, Chester A. Ray

Abstract—An inverse relation exists between omega-3 fatty acid intake and risk of cardiovascular disease development/mortality and sudden cardiac death in humans. Mechanisms underlying this cardioprotective effect are unknown, but could involve the autonomic nervous system. We tested the hypothesis that omega-3 fatty acid supplementation (“fish oil”) would reduce muscle sympathetic nerve activity (MSNA) at rest and attenuate increases during physiological stressors. MSNA (peroneal microneurography) was measured during rest, ischemic handgrip to fatigue (IHG), and a cold pressor test (CPT). Measurements were obtained before (PRE) and after (POST) 1 month of daily ingestion of either fish oil (experimental group, n=9) or olive oil capsules (control group, n=9). MSNA at rest was comparable PRE and POST in control (3 bursts/30 seconds) and experimental (4 bursts/30 seconds) subjects. IHG and CPT increased MSNA in both groups PRE and POST. MSNA, arterial blood pressure, and heart rate responses to the stressors were similar PRE and POST in the control group. In contrast, MSNA responses to IHG (4 bursts/30 seconds; P<0.05 for PRE and POST, respectively) and CPT (4 bursts/30 seconds; P<0.05) were augmented after omega-3 fatty acid supplementation whereas arterial blood pressure and heart rate responses were unchanged. These data indicate that 1 month of omega-3 fatty acid supplementation does not change MSNA at rest but augments sympathetic outflow to physiological stressors. The mechanism underlying augmented MSNA responses to physiological stressors after omega-3 fatty acid supplementation is unknown, but may involve impaired peripheral vasoconstriction. (Hypertension. 2004;44:732-738.)

Key Words: autonomic nervous system ■ blood pressure ■ fatty acids ■ cardiovascular diseases

A n inverse relation exists between omega-3 fatty acid intake and risk of cardiovascular disease development/mortality and sudden cardiac death in humans.1–8 The mechanisms underlying the cardioprotective effects of omega-3 fatty acids are likely to be multifactorial9–16 and may involve the autonomic nervous system. Data suggesting a link between omega-3 fatty acids and the autonomic nervous system include favorable associations between indices of cardiac autonomic outflow and omega-3 fatty acid intake.17,18 Additionally, sympathetic nervous system outflow may be reduced by omega-3 fatty acids as suggested by decreased plasma norepinephrine levels after increased dietary intake of fish or fish oils.19,20 Presently the effect of omega-3 fatty acid supplementation on directly recorded efferent sympathetic nerve activity at rest and in response to sympathoexcitatory stressors is unknown.

Accordingly, in the present study we tested the hypothesis that 1 month of omega-3 fatty acid supplementation would reduce muscle sympathetic nerve activity (MSNA) at rest and in response to physiological stressors. Moreover, we hypothesized that these effects on MSNA would attenuate the magnitude of the pressor response to physiological stressors. These data should expand our knowledge of how omega-3 fatty acids influence the autonomic nervous system and possibly reduce cardiovascular disease associated morbidity/mortality in humans.

Methods

Subjects
Eighteen young (age 18 to 35 years) subjects were studied. Subjects were normotensive, nonsmoking, nonobese (body mass index <27kg/m²), nonmedicated, and otherwise healthy based on medical history and physical examination.

Written informed consent was obtained on Pennsylvania State University College of Medicine Institutional Review Board-approved forms.

Experimental Design
This study was randomized, placebo-controlled, and double-blinded. Subjects were studied supine and fasted (12 hours) on 2 occasions separated by 1 month. The time of day of the testing differed between, but not within subjects.

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Protocol
After establishing an MSNA site, a 5-minute recording was obtained to quantify MSNA at rest. After obtaining these data, responses to physiological stressors was assessed in a randomized fashion. Fifteen minutes elapsed between stressors.

Ischemic Handgrip to Fatigue
After a 2-minute baseline period, subjects performed ischemic handgrip (IHG) at 30% of their maximal voluntary contraction to fatigue. Maximal voluntary contraction force was assessed in triplicate at the beginning of the protocol. IHG was stopped when target force could not be maintained for >3 seconds. IHG was performed to fatigue to allow comparisons at the same physiological time points.

Cold Pressor Test
After a 2-minute baseline period, subjects submerged their hand up to the wrist in a bucket of ice and water for 90 seconds. This was the cold pressor test (CPT).

Omega-3 Fatty Acids Supplementation
After obtaining the aforementioned measurements, subjects were given a container filled with capsules and instructed to ingest 10 capsules per day for 1 month. Containers were filled with 1-gram capsules containing either omega-3 fatty acids (Pharmacist’s Ultimate Health, Saint Paul, Minn; 300 mg eicosapentaenoic acid [EPA] and 200 mg docosahexaenoic acid [DHA]) or placebo (olive oil). A local compounding pharmacist assembled the placebo capsules. Efforts to increase/verify compliance to pill ingestion included review of subject pill ingestion diary, pill count, and phone-based contacts.

After this 1-month period, subjects returned to the laboratory and the experimental measurements were repeated in the same order as in testing before 1 month of oil ingestion. Subjects were asked not to ingest any capsules the day of testing after 1 month of oil ingestion.

Measurements
Multifiber recordings of MSNA were obtained by inserting a tungsten microelectrode in the peroneal nerve of the leg.21 Raw nerve recordings were amplified, filtered, full wave-rectified, and integrated to obtain mean voltage neurograms.

Arterial Blood Pressure and Heart Rate
Resting arterial blood pressure (BP) was determined (Dinamap XL, Johnson & Johnson) after a 5-minute period of rest (5 times at 1-minute interval). BP measurements during physiological stressors were made using a Finapres (Ohmeda). Heart rate was determined with ECG.

Blood Samples
Venous blood samples were obtained before and after the 1-month period. Analyses included blood lipids, C-reactive protein (using an immunoturbidimetric assay),23,24 and prostanoids (6-Keto-PGF_1α and thromboxane B_2).23,24

Data Analysis
Physiological data were displayed and recorded (MacLab 8e, AD-Instruments) at 400 Hz for later analysis.

Resting MSNA was quantified from 5-minute recordings as bursts per 30 seconds. Burst frequency is a highly reproducible method of reporting MSNA within the same subject across periods of weeks to months.33 Additionally, MSNA was quantified as the sum of the area under individual MSNA bursts (total MSNA: arbitrary units [au]/30 seconds). Quantifying MSNA in this manner is dependent on both burst frequency and the size of individual bursts. Importantly, the latter is influenced by system amplification and microelectrode position within the nerve. Neurograms are normalized by assigning the largest burst at rest an arbitrary amplitude of 1000 and a portion of the nerve recording in which neural silence occurs for ~5 seconds a value of zero. This normalization technique assumes that the largest bursts at rest represent an equal amount of neural outflow day-to-day and between subjects. Based on this assumption comparing absolute levels of total MSNA between subjects or across days in the same subject may not be appropriate. For this reason, we generally report the relative magnitude of sympathoexcitation to provide further additional support for the data derived from the burst frequency data (which are not dependent on such assumptions). This approach appears appropriate, because each individual subject’s baseline level of total MSNA will serve as a control level for the response to the stressors. Accordingly, responses to the physiological stressors were quantified as: (1) absolute burst frequency (bursts/30 seconds); (2) as change in burst frequency from baseline (Δ bursts/30 seconds); (3) as percent change from baseline in total MSNA (%Δ total MSNA); and (4) as the change in total MSNA (Δ au/30 seconds). BP was quantified as systolic, diastolic, and mean BP. Heart rate was determined at 30-second intervals and is reported as beats per minute.

Statistical Analysis
Differences in baseline subject characteristics were determined by t test, and repeated measures ANOVA was used to determine effects of the intervention. Statistical significance was established at P<0.05.

Results
Subject Characteristics
Subject characteristics in the experimental (omega-3 fatty acid) and control (olive oil) groups before and after the intervention are shown in the Table. Importantly, MSNA at rest (in any expression) and BP at rest were unchanged by either intervention.

Responses to Physiological Stressors
Autonomic and cardiovascular parameters were similar before each of the stressors before and after the intervention.

IHG to Fatigue
MSNA, BP, and heart rate increased (P<0.05) during IHG to fatigue before and after the intervention in experimental and control subjects (Figure 1). Increases in MSNA, BP, and heart rate were time-dependent and maximal in the final 30 seconds of IHG. In control subjects, MSNA (Δ burst frequency, Δ total MSNA, and %Δ total MSNA), mean BP, and heart rate responses to IHG were similar before and after the intervention (Figure 1). In contrast, MSNA responses were augmented in the experimental group after the intervention (Δ9±2 bursts/30 seconds, Δ355±83%, and Δ1463±360 au/30 seconds for burst frequency, %Δ total MSNA, and Δ total MSNA, respectively) compared with before the intervention (Δ4±2 bursts/30 seconds, Δ104±48%, and Δ585±264 au/30 seconds; P<0.05 for burst frequency and %Δ total MSNA and P=0.06 for Δ total MSNA) (Figure 1). Increases in mean BP (Δ34±6 versus Δ33±6 mm Hg) and heart rate (Δ22±4 versus Δ22±5 bpm) were similar before and after the intervention in the experimental group (Figure 1). Because of variations in total MSNA at baseline, the effects are less pronounced than when data are expressed as percent change (%Δ) in total MSNA and change (Δ) in total MSNA (Figure 1). The limitations associated with the expression of total MSNA have been discussed (see Methods). Collectively, these data indicate that IHG-induced increases in MSNA (burst frequency, and %Δ total MSNA), but not in
BP and heart rate, are augmented by 1-month ingestion of omega-3 fatty acids.

CPT
CPT increased MSNA and BP (P<0.05) in both groups before and after the intervention. In the control group, CPT-induced increases in MSNA (burst frequency, Δ total MSNA, and %Δ total MSNA), mean BP, and heart rate were not different before and after the intervention (Figure 2). Peak CPT-induced increases in MSNA (burst frequency) were greater (P<0.05) after omega-3 fatty acid supplementation (Δ4±1 versus Δ10±2 bursts/30 seconds; change from baseline for before and after the intervention, respectively). When MSNA was expressed as the absolute increase in total MSNA (Δ1206±300 versus Δ2021±426 au/30 seconds; change from baseline for before and after the intervention, respectively) and as the relative increase in total MSNA (Δ347±93 versus Δ520±175%) responses were greater after the intervention, but not statistically (Figure 2). Increases in MSNA during CPT elicited similar significant increases in mean BP (Δ19±2 versus Δ21±2 mm Hg) and heart rate (Δ8±1 versus Δ7±3 bpm) (Figure 2) before and after the intervention in the experimental group. Collectively, these data indicate that CPT-induced increases in MSNA burst frequency, but not in BP or heart rate, are augmented by 1-month ingestion of omega-3 fatty acids.

Discussion
The primary new finding from the present study is that 1-month ingestion of omega-3 fatty acids does not alter MSNA at rest, but does augment stress-induced increases. Despite the augmented MSNA responses to stress after ingestion of omega-3 fatty acids, the systemic pressor response was not altered, suggesting that the process by which increases in MSNA produce vasoconstriction may be impaired by omega-3 fatty acids.

Experimental data indicate that fish consumption and omega-3 fatty acid supplementation reduce morbidity/mortality associated with cardiovascular disease as well as risk of sudden cardiac death.8,26 The mechanisms underlying these effects could involve a number of physiological systems.27 One system that has received limited attention is the autonomic nervous system. In this context, experimental data suggest favorable associations between omega-3 fatty acid intake and cardiac autonomic outflow.17,18,28 Moreover, plasma norepinephrine levels, which provide a gross index of whole-body sympathetic outflow, have been shown to be reduced at rest after fish or fish oil intake in some,19,20 but not all studies.29 Our results using a direct and more powerful measure of sympathetic outflow (ie, MSNA) do not support these earlier findings19,20 or the hypothesis that omega-3 fatty acids reduce MSNA at rest in healthy young adults. It is possible that different results may be obtained if these measures were made in populations with elevated baseline levels (eg, older adults or in congestive heart failure).

Few data exist examining sympathetic nervous system responses to physiological stress after omega-3 fatty acid supplementation. Increases in plasma norepinephrine during hypovolemic stress are not altered by 4-week ingestion of

### Subject Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Olive Oil</th>
<th>Fish Oil</th>
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<tbody>
<tr>
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<td>4/5</td>
<td>—</td>
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<td>Age, y</td>
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<td>Height, cm</td>
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<td>Body mass, kg</td>
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<td>BMI, kg/m²</td>
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<td>MSNA, bursts/30 s</td>
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<td>Triglycerides, mg/dL</td>
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<td>C-reactive protein, mg/dL</td>
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<td>90±5</td>
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<tr>
<td>Thromboxane B2, pg/mL</td>
<td>169±10</td>
<td>152±11</td>
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<tr>
<td>IHG time to fatigue, s</td>
<td>210±13</td>
<td>238±24</td>
</tr>
</tbody>
</table>

Values are mean±SE. BMI indicates body mass index; BP, blood pressure; MSNA, muscle sympathetic nerve activity; au, arbitrary units; HDL, high-density lipoprotein; LDL, low-density lipoprotein; IHG, ischemic handgrip; Before, before 1 month of oil; After, after 1 month of oil. Interaction indicates P value of 2-way (group×time) repeated measures ANOVA interaction. *P<0.05 from before.
omega-3 fatty acids. However, although not statistically different, these responses were ~20% greater after the intervention. It is possible that use of a direct more powerful measure of sympathetic outflow (ie, MSNA) allowed us to observe an effect of omega-3 fatty acids on sympathetic responses to physiological stressors. However, because mechanisms mediating increases in sympathetic outflow during central hypovolemic stress and IHG and CPT differ, we cannot exclude the possibility that our results are specific to these stressors.

The mechanism(s) mediating the augmented MSNA response to the physiological stressors in the present study is unknown. Previously, 4- to 6-week ingestion of omega-3 fatty acids was shown to blunt forearm vasoconstrictor responses to intrabrachial infusion of norepinephrine. Extending these findings, it is possible that the augmented MSNA response to physiological stressors we observed was appropriate if end-organ responsiveness was impaired. For instance, during IHG the body increases MSNA in an attempt to increase perfusion pressure in the active limb (flow
error”) and systemic BP secondary to baroreflex resetting (“pressure error”). If forearm vascular responsiveness to increases in MSNA were attenuated, then greater increases in MSNA would be necessary to elicit the same pressor effect and to appropriately respond to these error signals. Data from the IHG trial are consistent with this concept. Specifically, the systemic pressor response to IHG was not influenced by ingestion of omega-3 fatty acids, but the reflex increase in MSNA was. In lieu of a change in central hemodynamic responses, these data suggest that end-organ responsiveness was impaired by omega-3 fatty acids. Future studies measuring limb blood flow and central hemodynamics before and after omega-3 fatty acid supplementation are needed to definitively address this question.

If end-organ responsiveness was impaired (ie, the ability of skeletal muscle to vasoconstrict in response to an increase in MSNA) by omega-3 fatty acid supplementation, then it is possible that factors locally produced contributed to this effect. Two local factors that may be influenced by omega-3 fatty acid supplementation and that may impair vasoconstrictor responses are nitric oxide33 and prostanoids.34 We have no measure, index, or assay of nitric oxide in the present study.

Figure 2. Responses to cold pressor test (CPT) before (Pre) and after (Post) 1-month intake of omega-3 fatty acids (experimental) or olive oil (control). All data are derived from the 30-second period before each time point and are reported as change from baseline. Note the Post MSNA response to CPT is greater (∆MSNA) in the experimental group. In contrast, the MAP and HR responses were not different. Olive oil intake did not affect any of the physiological responses. *P<0.05.
Therefore, we cannot determine if nitric oxide production was increased after the intervention or if it contributed to any of our findings. Additionally, prostaglandins may have influenced our results. However, we were unable to document any effect of omega-3 fatty acids on several markers of prostanooid synthesis (ie, 6-Keto-PGF1α, and thromboxane B2) (Table). These findings suggest that our results may not be dependent on changes in prostanooids. However, to definitively determine the role of prostanooids or nitric oxide on the present findings would require repeating the present study while inhibiting cyclooxygenase and/or nitric oxide synthase.

These previous discussions suggest that postsynaptic mechanisms explain augmented MSNA responses to physiological stressors. However, modulators of neurovascular nor-epinephrine levels, such as release, uptake, or spillover, cannot be excluded. If altered, these could reduce end-organ exposure to neurotransmitter, resulting in a reduced vasoconstrictor signal. Consistent with this concept, DHA enhances purine release in rats, which may act to inhibit sympathetic nerve terminal release of norepinephrine.20 This reduced norepinephrine release would necessitate a greater neural impulse to produce the same neurovascular concentration of norepinephrine. Thus, we cannot exclude the possibility that factors associated with modulation of neurovascular norepinephrine levels contributed to our findings.

Several limitations are associated with the present study. First, gender balance was not even between groups. However, we are not aware of any data suggesting that gender influences responses to omega-3 fatty acids. Second, no measure of peripheral blood flow was obtained. Therefore, we cannot be certain that for a given change in MSNA, end-organ response was impaired by omega-3 fatty acids. Third, the number of subjects studied was small. Fourth, we examined sympathetic outflow innervating skeletal muscle arterioles. Because sympathetic outflow is highly heterogeneous, it is possible that sympathetic outflow to other organs may not have been similarly affected by omega-3 fatty acids. In this regard, it may be important to examine sympathoexcitatory responses to other organs important in mediating stress responses (eg, cardiac) in future studies.

Lastly, it is important to emphasize that the most consistent effect of omega-3 fatty acid supplementation on MSNA responses to physiological stressors was observed when MSNA was expressed as burst frequency. The other measures of MSNA (%Δ total MSNA and Δ total MSNA) produced similar results to the burst frequency data during IHG, but less clear results during CPT. Specifically, changes in total MSNA at fatigue during IHG were greater after omega-3 fatty acid supplementation when data were expressed as %Δ total MSNA (P<0.05) and tended to be greater when expressed as Δ total MSNA (P=0.06), entirely consistent with the burst frequency data. These augmented MSNA responses after omega-3 fatty acid supplementation were not as consistently apparent during the CPT. Despite the fact that the MSNA burst frequency data indicated an augmented response to the CPT after omega-3 fatty acids, the total MSNA data (ie, %Δ total MSNA or Δ total MSNA) were not as clear (Figure 2). Based on this observation, it could be argued that MSNA responses are not augmented after omega-3 fatty acids, particularly during the CPT trial. We are uncertain as to why there is a partial disconnect between the burst frequency and total activity data during the CPT. We can only speculate that these discrepancies are associated with inherent methodological limitations associated with reporting changes in total MSNA (either as Δ total MSNA or %Δ total MSNA) in humans using microneurography especially when measured on separate days (see Methods). Ultimately gaining insight into end-organ responsiveness requires that events occurring within the neurovascular junction be examined. In this regard, the use of burst frequency as a surrogate for events taking place in the neurovascular junction in humans is supported by the strong association between changes in burst frequency and changes in interstitial norepinephrine concentrations in humans.35 However, we cannot entirely discount the alternative interpretation that responses were not augmented but believe that our conclusions are valid based on the available data. At minimum, our data demonstrate that 1 month of omega-3 fatty acid supplementation does augment MSNA responses to physiological stressors when burst frequency is considered.

Perspectives

These data suggest that 1 month of omega-3 fatty acid supplementation augments MSNA responses to several distinct physiological stressors but does not alter resting levels. The augmented MSNA response to physiological stressors after omega-3 fatty acid supplementation may be caused by an impaired ability of MSNA to elicit vasoconstriction. This impairment in vasoconstriction is suggested by the fact that the augmented MSNA response after omega-3 fatty acid supplementation was not associated with an augmented systemic pressor response to the stressors. Impaired vasoconstriction could involve numerous factors, including blunted end organ responsiveness (ie, altered adrenergic receptor sensitivity), alterations in exposure of the end organ to neurotransmitter (ie, norepinephrine), or altered local production of factors that may modify vasoconstriction generations (eg, nitric oxide or prostanooids). Further studies directly examining these specific mechanisms appear warranted and may expand our knowledge of how omega-3 fatty acids reduce cardiovascular disease-associated risks in humans.

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