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Fish oil and neurovascular reactivity to mental stress in humans

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[1]*Department of Kinesiology and Integrative Physiology, Michigan Technological University, Houghton, Michigan;* [2]*Department of Pediatrics, New York Medical College, Valhalla, New York; and* [3]*Department of Anesthesiology, Mayo Clinic, Rochester, Minnesota*

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Carter JR, Schwartz CE, Yang H, Joyner MJ. Fish oil and neurovascular reactivity to mental stress in humans. *Am J Physiol Regul Integr Comp Physiol* 304: R523–R530, 2013. First published February 13, 2013; doi:10.1152/ajpregu.00031.2013.—Omega-3 fatty acids found in fish oil have been suggested to protect against cardiovascular disease, yet underlying mechanisms remain unclear. Despite the well-documented link between mental stress and cardiovascular risk, no study has examined neural cardiovascular reactivity to mental stress after fish oil supplementation. We hypothesized that fish oil would blunt the blood pressure, heart rate (HR), and muscle sympathetic nerve activity (MSNA) responsiveness to mental stress and/or augment limb vasodilation associated with mental stress. Blood pressure, HR, MSNA, forearm vascular conductance (FVC), and calf vascular conductance (CVC) responses were recorded during a 5-min mental stress protocol in 67 nonhypertensive subjects before and after 8 wk of fish oil ($n = 34$) or placebo supplementation ($n = 33$). Fish oil blunted HR reactivity to mental stress (group × condition × time interactions, $P = 0.012$) but did not alter blood pressure reactivity to mental stress (interactions, $P > 0.05$). Fish oil blunted total MSNA reactivity to mental stress (interaction, $P = 0.039$) but did not alter MSNA burst frequency and burst incidence reactivity (interactions, $P > 0.05$). Finally, fish oil significantly blunted CVC reactivity to mental stress (interaction, $P = 0.013$) but did not alter FVC reactivity (interaction, $P > 0.05$). In conclusion, 8 wk of fish oil supplementation significantly attenuated both HR and total MSNA reactivity to mental stress and elicited a paradoxical blunting of calf vascular conductance. These findings support and extend the growing evidence that fish oil may have positive health benefits regarding neural cardiovascular control in humans.

omega-3 fatty acids; blood pressure; sympathetic nerve activity; autonomic function; cardiovascular reactivity

A GROWING BODY OF EVIDENCE suggests that the omega-3 fatty acids found in fish oil protect against cardiovascular disease. Fish oil has been shown to improve vascular function through a variety of factors including reduced triglyceride levels (20), decreased growth rate of atherosclerotic plaques (13), and reduced blood pressure (34). The antihypertensive effect of fish oil remains controversial, but meta-analyses conclude that high intake of fish oil appears to lower blood pressure, especially in hypertensive subjects (2, 15, 34). Mechanisms responsible for fish oil-induced reductions of blood pressure have not been completely elucidated, but reduction in sympathetic nerve activity has been suggested as a potential contributor. To date, only two studies have employed microneurographic techniques to directly assess postganglionic sympathetic neural traffic before and after fish oil supplementation (8, 31). Monahan et al. (31) reported no change in resting muscle sympathetic nerve activity (MSNA) after 1 mo of fish oil in normotensive subjects. More recently, our laboratory (8) supported and extended the findings of Monahan et al. (31) by demonstrating no change in resting MSNA after fish oil in both normotensive or prehypertensive individuals. However, a more detailed analysis revealed that fish oil was associated with modest sympathoinhibition in individuals with higher resting heart rates (HR) (8), a finding consistent with a recent meta-analysis suggesting resting HR as an important variable regarding the effectiveness of fish oil supplementation (35).

In addition to examining resting MSNA, Monahan et al. (31) also examined sympathetic neural responsiveness to two classic sympathoexcitatory maneuvers. Specifically, Monahan et al. (31) reported an augmented MSNA response to cold pressor test (CPT) and ischemic handgrip to fatigue (IHG). In contrast, Delarue et al. (10) recently demonstrated that supplementation with fish oil inhibited concentrations of circulating catecholamines during mental stress in healthy men, but sympathetic neural and limb vascular responses were not measured. The divergent sympathetic responses to fish oil supplementation between Monahan et al. (31) and Delarue et al. (10) suggest that the type of sympathetic stressor (IHG/CPT vs. mental stress) and/or the sympathetic assessment technique (MSNA vs. plasma catecholamines) may be important. To date, the effects of fish oil on MSNA and limb vascular reactivity to mental stress have not been investigated.

In addition to evoking altered neural control, mental stress elicits a well-documented forearm vasodilation (3, 4). This vasodilatory response has been implicated as an important vascular adjustment relevant to hypertension (6, 39, 41). Omega-3 fatty acids have been shown to enhance forearm vasodilation in response to acetylcholine, an endothelium-dependent vasodilatory agent (32, 33). Such pharmacodissection data are novel and relevant, but what effect does fish oil have on the vasodilation associated with the nonpharmacological mental stress intervention? To date, no studies have investigated mental stress-induced forearm vasodilation after fish oil supplementation. Such a study could be of significance to cardiac heart failure (40) and hypertensive (39) subjects, two populations that consistently demonstrate blunted forearm vasodilation during mental stress.

Therefore, the purpose of the present study was to determine MSNA and limb vascular reactivity to mental stress before and after omega-3 fatty acid supplementation. We hypothesized that fish oil would blunt the blood pressure, HR, and MSNA responsiveness to mental stress and/or augment vasodilatory responses to mental stress. Identifying the mechanism(s) by which omega-3 fatty acids lower blood pressure remains a critical gap in the field of hypertension research.

METHODS

Subjects. Sixty-seven nonhypertensive (resting blood pressure < 140 systolic/90 diastolic) subjects (fish oil: 24 men, 10 women; placebo: 22 men, 11 women) age 24 ± 1 yr participated in this study.

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Resting variables for these 67 subjects have been previously reported (8). All subjects signed an informed consent and refrained from caffeine, alcohol, and exercise for at least 12 h before testing. Exclusion criteria for participants included smoking, diabetes, autonomic dysfunction, and use of blood pressure medication. This study was approved by the Michigan Technological University Institutional Review Board.

Experimental protocol. All subjects were randomly assigned into this double-blind, placebo-controlled investigation and were tested before (pre) and after (post) 8 wk of fish oil or placebo (olive oil) supplementation (parallel administration of fish oil and placebo). Subjects reported to the Integrative Physiology Laboratory at Michigan Technological University at the same time of day for 3 consecutive days to establish resting blood pressure during the pre- and posttreatment sessions. After the seated blood pressure recordings on *day 3* of the pretreatment, subjects were instrumented for the mental stress protocol, which consisted of a 10-min baseline and 5 min of mental stress. Mental stress was elicited via serial subtraction (i.e., mental arithmetic). Briefly, subjects continuously subtracted the number 6 or 7 from a two- or three-digit number verbally and were encouraged by an investigator to subtract as quickly as possible. The subtraction number (i.e., 6 or 7) was randomized between the pre- and posttrials. Immediately after the mental stress protocol, subjects were asked to report rating of perceived stress using a standard 0–4 perceived stress scale (5).

Upon completion of the pretreatment testing, subjects were randomly assigned into the fish oil or placebo (olive oil) group. Participants ingested 9 g/day of fish oil pills (1.6 g eicosapentaenoic acid, 1.1 g docosahexaenoic acid) or 9 g/day of placebo pills for 8 wk. The fish oil dosage and the selection of olive oil as the placebo were based on a literature review of double-blinded, placebo-based studies examining the influence of fish oil on blood pressure in humans. A pill diary and regular email and phone reminders were issued to the subjects to track compliance according to the study terms. Subjects were asked to maintain current diet and exercise habits during the 8-wk supplementation period. After treatment, subjects returned to the laboratory for posttesting and completed the same protocol as pretesting. Two subjects completed the complete pretesting but only were able to complete for the seated resting measurements. Additionally, one subject was unable to complete the postmental stress protocol. Accordingly, hemodynamic responses to mental stress are reported in 64 total subjects ($n = 32$ fish oil, $n = 32$ placebo).

Measurements. HR was measured using a three-lead electrocardiogram. Arterial blood pressure was measured utilizing two different methods. Seated resting blood pressures were obtained using the IntelliSense automated sphygmomanometer (model HEM-907XL, Omron Healthcare, Bannockburn, IL). Seated resting recordings were taken three consecutive times each day, separated by 1 min and following 5 min of seated rest. Importantly, all pre- and posttreatment blood pressure measurements were recorded at the same time of day to limit diurnal blood pressure variations as a potential confounder (38). Beat-to-beat arterial pressure was recorded using the Finometer (Finapres Medical Systems, Amsterdam, The Netherlands) on the middle finger throughout autonomic testing. The Finometer is an ideal technique for measuring beat-to-beat changes in arterial pressure but is not accurate and reproducible in measuring absolute values. Therefore, we used the seated resting brachial arterial pressure as absolute resting values and the Finometer for recording relative changes during mental stress and recovery. Blood pressures are reported as systolic (SAP), diastolic (DAP), and mean (MAP) arterial pressure.

Multifiber recordings of MSNA were recorded using the microneurography technique. Briefly, a tungsten microelectrode was inserted into the common peroneal nerve located in the popliteal region of the knee or at the base of the fibular head of the lower leg. A reference electrode was inserted subcutaneously 2–3 cm away from the recording electrode. Both electrodes were connected to a preamplifier and amplifier. The signal was amplified 80,000 times, band-pass filtered (700–2,000 Hz) and integrated (time constant, 0.1 s) to obtain a mean voltage display of nerve activity. MSNA was determined by observing spontaneous multifiber bursts of activity and confirmed by having the subject perform end-expiratory apnea with a resultant increase in MSNA. The signal was distinguished from skin sympathetic nerve activity by performing auditory stimulation with no subsequent neural reaction. Complete MSNA responses to mental stress are available in 29 subjects (12 fish oil, 17 placebo). Because of shifts in neurogram, measurements of total MSNA are available in 23 subjects (9 fish oil, 14 placebo).

Forearm (FBF) and calf (CBF) blood flow were measured using venous occlusion plethysmography (D. E. Hokanson, Bellevue, WA). Occlusion cuffs were placed around the left upper arm, wrist, thigh, and ankle. The wrist and ankle cuffs were inflated to 220 mmHg to temporarily prevent circulation to the hand and foot. The arm and thigh cuffs were then inflated to 60 mmHg for 7 s and deflated for 8 s (i.e., 4 readings per min). Inflation of the collecting cuffs allows arterial blood flow into the forearm and calf but prevents venous flow return. Strain gauges were placed around the greatest circumference of the forearm and calf allowing for direct measurements of changes in arm and leg circumference. Technical issues and artifact during mental stress prevented the analysis of forearm and calf blood flow in several subjects. We were able to record forearm measurements in 29 fish oil subjects and 26 placebo subjects, while in the calf we recorded measurements in 28 fish oil and 25 placebo subjects during mental stress.

Table 1. *Baseline values before (pre) and after (post) 8 wk of fish oil or placebo*

	Fish Oil		Placebo		
	Pre	Post	Pre	Post	Interaction *P* Value
SAP, mmHg	117 ± 2	115 ± 2	115 ± 2	114 ± 2	0.46
DAP, mmHg	67 ± 1	67 ± 1	69 ± 1	69 ± 1	0.72
MAP, mmHg	84 ± 1	83 ± 1	84 ± 1	84 ± 2	0.59
HR, beats/min	72 ± 2	72 ± 2	75 ± 2	75 ± 2	0.61
MSNA, bursts/min	11 ± 1	10 ± 1	12 ± 1	12 ± 2	0.57
MSNA, bursts/100 hb	17 ± 2	16 ± 2	18 ± 2	18 ± 2	0.84
FBF, units	3.1 ± 0.2	3.1 ± 0.2	2.6 ± 0.2	2.8 ± 0.3	0.48
FVC, 100×U/mmHg	3.3 ± 0.3	3.5 ± 0.3	2.8 ± 0.2	3.2 ± 0.3	0.63
CBF, units	2.4 ± 0.2	2.6 ± 0.2	2.3 ± 0.1	2.4 ± 0.2	0.60
CVC, 100×U/mmHg	2.5 ± 0.2	2.9 ± 0.2	2.4 ± 0.1	2.8 ± 0.2	0.54

Values are means ± SE ($n = 34$ for fish oil, and $n = 33$ for placebo unless noted). SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial blood pressure; HR, heart rate; MSNA, muscle sympathetic nerve activity ($n = 27$ for fish oil and $n = 27$ for placebo); hb, heartbeats; FBF, forearm blood flow ($n = 32$ for fish oil and $n = 31$ for placebo); FVC, forearm vascular conductance; CBF, calf blood flow ($n = 30$ for fish oil and $n = 27$ for placebo); CVC, calf vascular conductance.

Data analysis. Data were recorded using WinDaq/Pro data acquisition software (DATAQ Instruments, Akron, OH), and imported into WinCPRS (Absolute Aliens, Turku, Finland) software package for analysis. R-waves from the electrocardiogram were detected and marked in the time series. Integrated bursts of MSNA were detected as a 3:1 burst-noise ratio within a search window of 0.5 s based on an average expected burst peak latency of 1.3 s following the previous R-wave. Spontaneous bursts of MSNA were normalized to the average burst size during baseline and designated a value of 100 arbitrary units. Sympathetic bursts of activity were expressed as burst frequency (bursts/minute), burst incidence (bursts/100 heartbeats), and total activity (arbitrary units). Total MSNA was determined as the burst activity multiplied by the average normalized area under the burst. This analysis accounts for the change in the size of the burst per minute. Blood flow data were analyzed using the NIVP3 software (D. E. Hokanson, Bellevue, WA). Percent changes in flow per time were analyzed in both the forearm and calf. Vascular conductance was calculated as blood flow divided by MAP in both the forearm (FVC) and calf (CVC).

Statistical analysis. Data were analyzed using commercial software SPSS 18.0 (SPSS Chicago, IL). Baseline values were compared using a 1-between (Group: fish oil vs. placebo) by 1-within (Condition: pre- vs. posttreatment) repeated measures analysis of covariance (ANCOVA), with resting MAP and resting HR as covariables. Responses to mental stress were analyzed using a 1-between (Group: fish oil vs. placebo) by 2-within (Condition: pre- vs. posttreatment; Time: baseline vs. mental stress) repeated measures ANCOVA, with resting MAP and resting HR as covariables. This statistical approach (i.e., consideration of pretreatment blood pressure and HR) is consistent with our previous study investigating the effects of fish oil on resting neural and cardiovascular control (8). Significant group $\times$ condition $\times$ time interactions were probed further using post hoc least significant difference pairwise comparisons. Mauchly's test of sphericity was performed for all ANCOVA analyses to test for differences between variances. A significant finding ($P < 0.05$) led to the use the Huynh-Feldt (sphericity values >0.75) or Greenhouse-Geisser (sphericity values <0.75) correction factors.

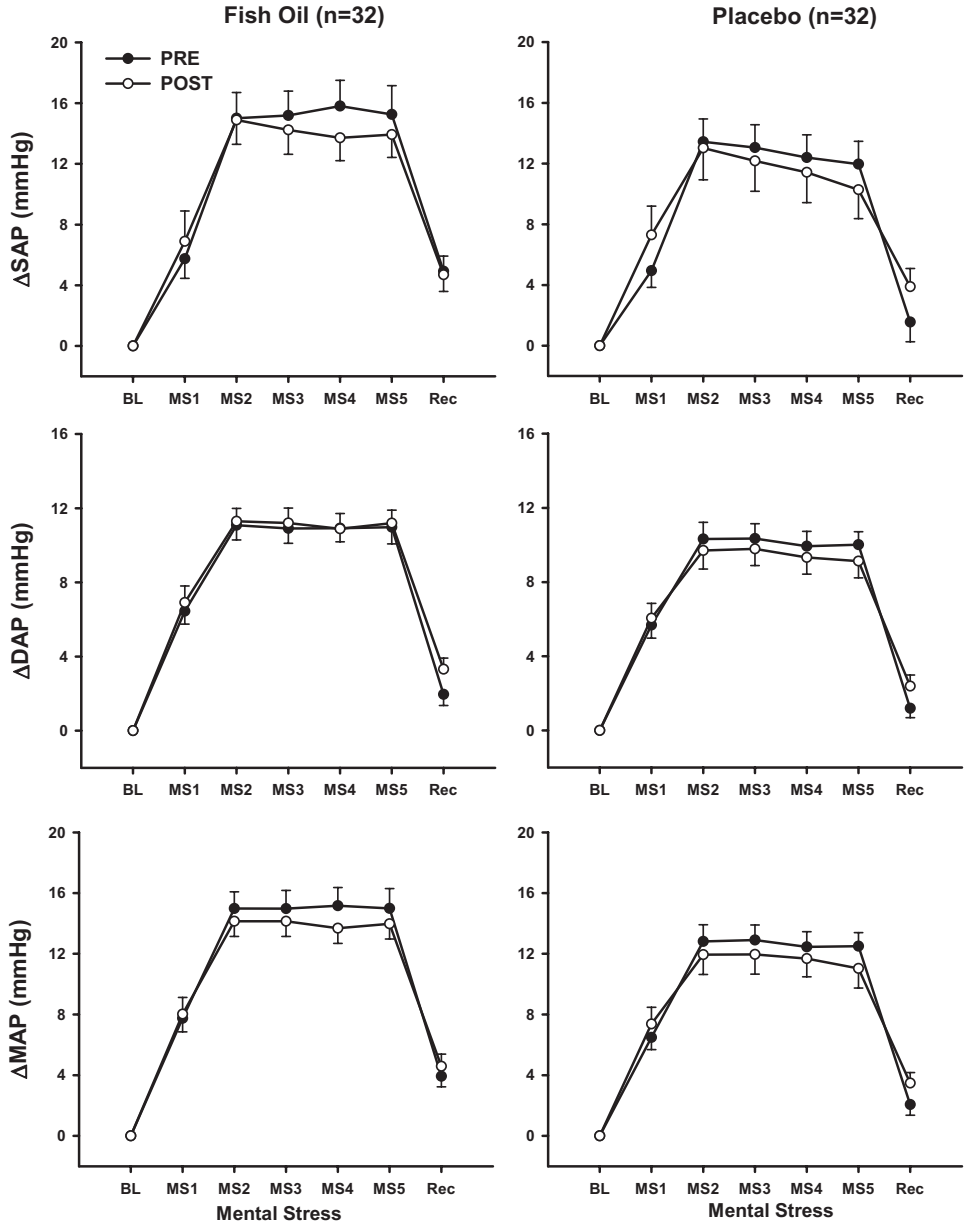

Fig. 1. Changes (Δ) in systolic (SAP), diastolic (DAP), mean (MAP) arterial pressures during mental stress fish oil or placebo supplementation. Mental stress significantly increased SAP, DAP, and MAP ($P < 0.001$), but these increases were not altered by fish oil (group $\times$ condition $\times$ time interactions; $P > 0.340$).

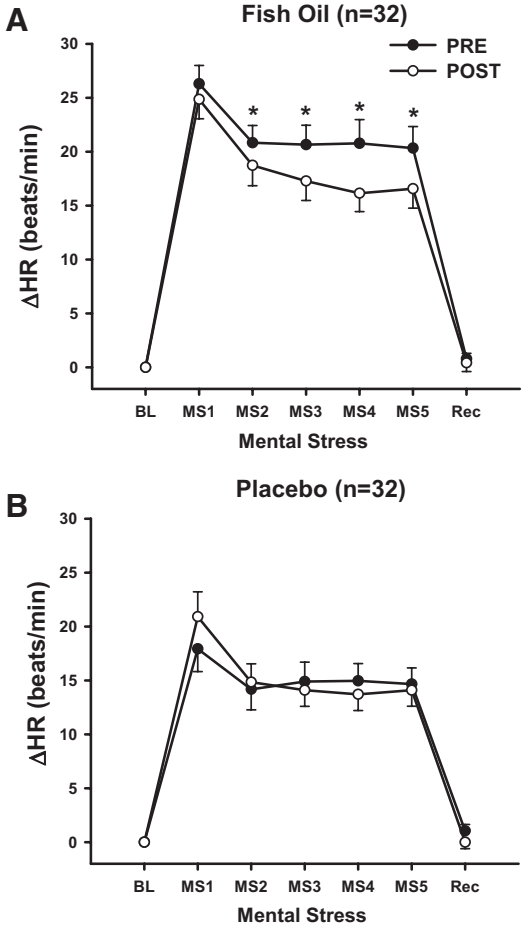

Fig. 2. Changes (Δ) in heart rate (HR) during mental stress pre- and post-fish oil or placebo supplementation. Mental stress significantly increased HR ($P < 0.001$), but these increases were blunted by fish oil (group × condition × time interaction; $P = 0.012$). *$P < 0.05$ vs. corresponding post value.

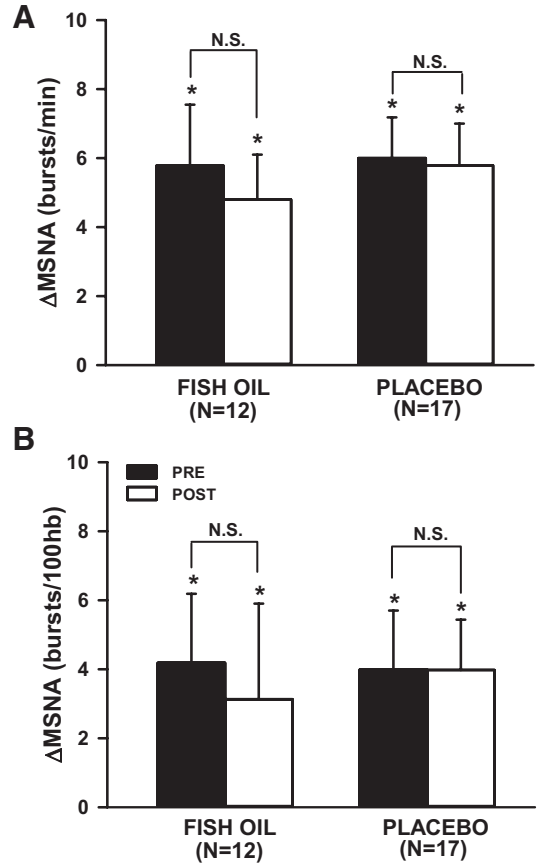

Fig. 3. Changes (Δ) in muscle sympathetic nerve activity (MSNA), expressed as burst frequency (bursts/minute) and burst incidence (bursts per 100 heartbeats), during mental stress pre- and post-fish oil or placebo supplementation. Fish oil did not alter MSNA burst frequency or incidence reactivity to mental stress. *Significant increase during mental stress, $P < 0.05$; N.S., no significance between pre- and posttrials.

All data expressed as means ± SE. Hemodynamic responses to mental stress are presented as 5-min averages in results text and 1-min averages in figures to provide more detail on differences between groups and conditions. For both MSNA and limb vascular data, there would occasionally be a missing data point during 1 min due to random artifact (i.e., movement of hand/foot and/or tensing of lower leg) that limited minute-by-minute analysis, thus 5-min averages are presented to maximize statistical power. Importantly, if there was a missing data point for 1 min in the pretreatment mental stress, we deleted the subsequent data point in the posttreatment (and vice versa); this ensured appropriate comparisons. This procedure was performed before any statistical analysis to avoid bias. Finally, it is important to note that two investigators independently confirmed there were no visible shifts in the MSNA neurograms for the comparisons of total MSNA.

RESULTS

Resting baseline. Table 1 depicts neural and cardiovascular values during resting baseline. SAP, DAP, and MAP are reported as the 3-day seated average (per methods description), while remaining variables are reported as the 5-min supine rest. Fish oil did not significantly alter any of the resting variables measured ($P > 0.05$). The interactions of these main resting variables with the covariables (i.e., pretreatment MAP and HR) have been previously reported and discussed (8).

Hemodynamic reactivity to mental stress. Figure 1 demonstrates that mental stress significantly increased SAP (fish oil, Δ13 ± 2 mmHg vs. Δ13 ± 2 mmHg; placebo, Δ11 ± 1 vs. Δ11 ± 2 mmHg; time, $P < 0.001$, pre- and posttreatment,

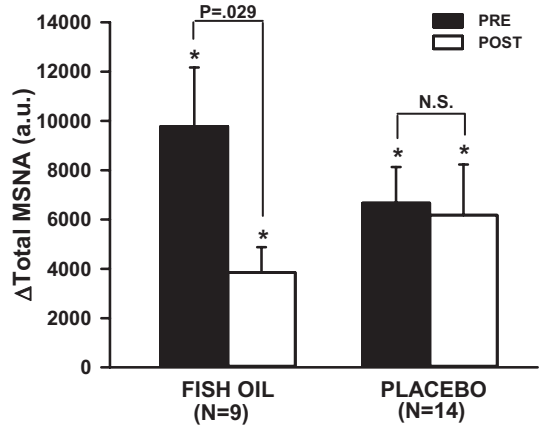

Fig. 4. Changes (Δ) in muscle sympathetic nerve activity (MSNA), expressed as total MSNA, during mental stress pre- and post-fish oil or placebo supplementation. Fish oil significantly blunted total MSNA reactivity to mental stress. *Significant increase during mental stress, $P < 0.05$; N.S., no significance between pre- and posttrials.

respectively), DAP (fish oil, Δ10 ± 1 vs. Δ10 ± 1 mmHg; placebo, Δ9 ± 1 vs. Δ9 ± 1 mmHg; time, $P < 0.001$), and MAP (fish oil, Δ13 ± 1 vs. Δ13 ± 1 mmHg; placebo, Δ11 ± 1 vs. Δ11 ± 1 mmHg; time, $P < 0.001$). These blood pressure responses were not different between the fish oil and placebo groups (group × condition × time interactions; $P > 0.340$). In contrast, Fig. 2 shows that HR reactivity to mental stress was attenuated after fish oil (Δ22 ± 2 vs. Δ19 ± 2 beats/min, $P = 0.002$) but not placebo (Δ15 ± 2 vs. Δ16 ± 2 beats/min, $P = 0.801$) supplementation (group × condition × time interaction; $P = 0.012$).

Sympathetic neural reactivity to mental stress. As depicted in Fig. 3, mental stress significantly increased MSNA burst frequency (fish oil, Δ6 ± 2 vs. Δ5 ± 1 bursts/min; placebo, Δ6 ± 1 vs. Δ6 ± 1 bursts/min; time, $P < 0.001$, pre- and posttreatment, respectively) and MSNA burst incidence (fish oil, Δ4 ± 2 vs. Δ3 ± 3 bursts/100 heartbeats; placebo, Δ4 ± 2 vs. Δ4 ± 2 bursts/100 heartbeats; time, $P < 0.001$). However, there were no differences between the fish oil and placebo groups (group × condition × time interactions, $P > 0.350$). In contrast, Fig. 4 shows that total MSNA response to mental stress was reduced by fish oil (Δ9,628 ± 2,407 to Δ3,830 ± 1,029 arbitrary units, $P = 0.029$) but not placebo (Δ6,860 ± 1,463 to Δ6,302 ± 2,042 arbitrary units, $P = 0.756$) treatment (group × condition × time interaction, $P < 0.05$). Figure 5 depicts a representative neurogram highlighting the blunted increase of total MSNA, but not MSNA burst frequency.

Limb vascular reactivity to mental stress. Figure 6 shows that mental stress increased FVC (fish oil, Δ69 ± 10% vs. Δ57 ± 11%; placebo, Δ45 ± 8% vs. Δ36 ± 8%; time, $P < .001$, pre- and posttreatment, respectively), but there were no significant differences between groups (group × condition × time interaction, $P > 0.800$). In contrast, the CVC reactivity to mental stress was blunted by fish oil (Δ25 ± 7% vs. Δ10 ± 5%; $P < 0.05$,) but not placebo (Δ11 ± 5% vs. Δ10 ± 6% post) treatment (group × condition × time interaction, $P < 0.01$). Perceived stress levels were not different pre- and post-fish oil (2.8 ± 0.1 vs. 2.8 ± 0.1 arbitrary units) or placebo (2.6 ± 0.1 vs. 2.8 ± 0.1 arbitrary units) (group × condition × time interaction, $P = .381$).

DISCUSSION

The current investigation offers several novel and important insights regarding the influence of omega-3 fatty acids on neural and cardiovascular function. First, consistent with our hypothesis we demonstrated that fish oil attenuated the HR reactivity to mental stress. In contrast, fish oil did not attenuate blood pressure reactivity to mental stress as predicted. We will discuss the significance of these findings as it relates to the cardiovascular reactivity hypothesis. Second, fish oil did not alter MSNA reactivity quantified as burst frequency or incidence. However, total MSNA reactivity to mental stress was significantly blunted by fish oil. Thus our data suggest that fish oil may influence MSNA recruitment strategies during mental stress; this could be highly relevant to cardiovascular disease models, particularly hypertension. Third, contrary to our initial hypothesis, limb vasodilation to mental stress was not augmented by fish oil. In fact, calf vasodilation during mental stress was actually blunted by fish oil. We believe these findings can be explained by the variety of factors that influence limb vascular reactivity to mental stress, particularly the reported blunted epinephrine reactivity to mental stress after fish oil (10). Overall, this study provides important mechanistic insight regarding the effects of fish oil on neurovascular reactivity.

The cardiovascular reactivity hypothesis postulates that hemodynamic responses to acute stressors, such as mental stress, provide predictive insight regarding future risk of cardiovascular disease. In fact, large-scale longitudinal studies have reported that heightened cardiovascular reactivity to mental stress is linked to an increased risk of developing hypertension

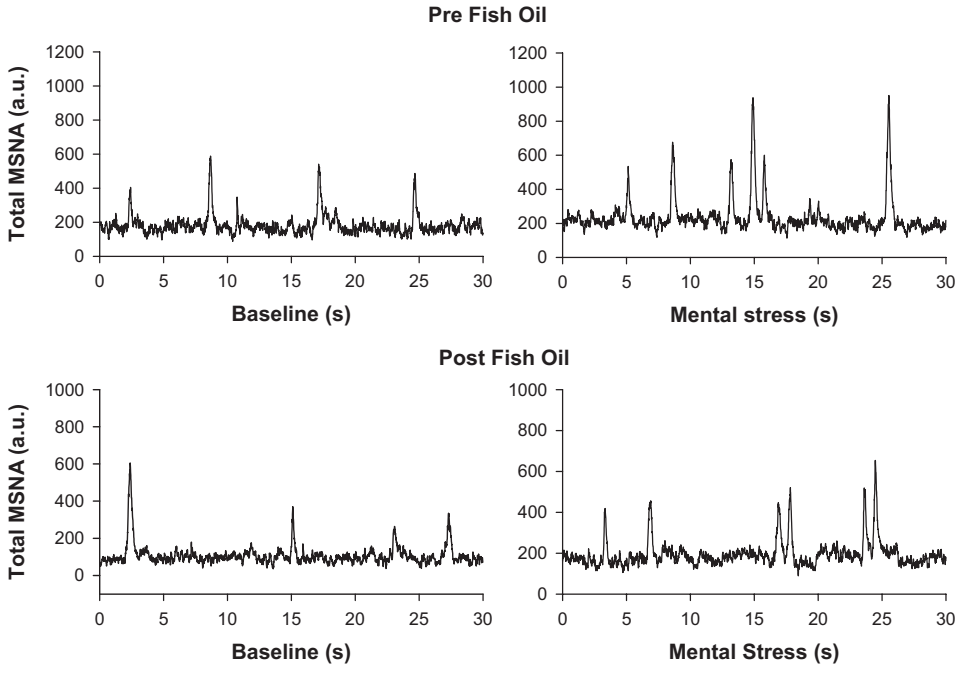

Fig. 5. Representative neurogram of one subject pre- and post-fish oil. Mental stress increased MSNA burst frequency, and these increases were similar before and after supplementation (consistent with Fig. 3). In contrast, increases in the amplitude/area of sympathetic bursts, which contribute to total MSNA, were blunted after fish oil (consistent with Fig. 4).

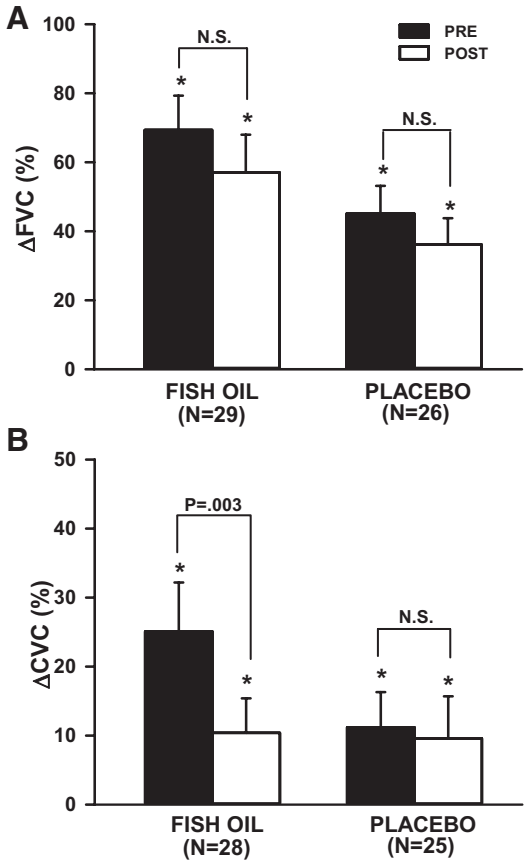

Fig. 6. Changes (Δ) in forearm vascular conductance (FVC) and calf vascular conductance (CVC) during mental stress pre- and post-fish oil or placebo supplementation. *Significant increase during mental stress, $P < 0.05$; N.S., no significance between pre- and posttrials.

(9, 28, 30, 42, 44, 46). Moreover, smaller observation studies report that both prehypertensive/hypertensive adults (21, 29, 39, 41), as well as subjects with a family history of hypertension (36, 45), demonstrate augmented cardiovascular reactivity to mental stress. What remains controversial is whether this augmented reactivity to mental stress has a causal relationship with cardiovascular disease or is simply a marker of future risk without any causal role. Regardless, investigations examining cardiovascular reactivity to mental stress and potential pharmacological and/or nonpharmacological therapies aimed at reducing this reactivity are important to the fields of hypertension and other cardiovascular disease research.

The present study provides new evidence that fish oil reduces HR reactivity to mental stress. Our laboratory has extensively examined neural and cardiovascular reactivity to mental stress. We have previously highlighted that although MSNA responsiveness can be variable (7), HR and blood pressure reactivity are remarkably consistent; most of our studies consistently report increases in HR of 15–20 beats/min and increases in blood pressure of 10–15 mmHg. Other laboratories using mental arithmetic to examine neural cardiovascular reactivity also report similar reactivity (1, 5, 19, 24), and there are several test-retest studies demonstrating strong within-subject cardiovascular reproducibility (23, 43). This reproducibility was observed within our placebo, lending credibility to the reported reduction of the HR reactivity with fish oil. Moreover, the sample sizes for the HR and blood pressure reactivity were robust to meet our goals for MSNA reactivity recordings. Overall, we are confident in our findings that fish oil attenuated HR reactivity to mental stress.

While we did not observe a similar attenuation of the blood pressure reactivity, a recent study did observe blunted blood pressure reactivity after fish oil (16). More specifically, Ginty and Conklin (16) recently reported that MAP reactivity to mental stress was attenuated after 21-day fish oil supplementation compared with placebo. Interestingly, the authors did not report an attenuated HR reactivity as observed in the present study, but there was a trend ($P = 0.28$) for reduced HR reactivity after fish oil (16). It is not entirely clear why our study demonstrated blunted HR reactivity without changes to MAP reactivity, and their study demonstrated the opposite. It is important to remember that while HR reactivity contributes to MAP reactivity, the blood pressure reactivity is also influenced by changes in stroke volume and total peripheral resistance (TPR). We do not have definitive measurements of either, and we explain shortly why we refrained from ModelFlow estimates. Nevertheless, it is not obligatory to see parallel changes in HR and MAP reactivity to mental stress after interventions. Additionally, differences between Ginty and Conklin (16) and the present study could relate to differences in subject demographics (much different male-to-female ratios between studies), differences in placebo (olive oil vs. corn oil), or differences in fish oil dosages and eicosapentaenoic acid/docosahexaenoic acid ratios. Regardless, the important piece is that both randomized, placebo-controlled studies independently observed some attenuation of cardiovascular reactivity to mental stress after fish oil. Collectively, they support the overarching concept that fish oil has certain cardiovascular benefits and suggest one potential mechanism for this benefit.

To date, only one study has examined the influence of omega-3 fatty acids on sympathetic reactivity to mental stress in humans. This is relevant because sympathetic outflow and reactivity play a key role in HR reactivity and limb vascular reactivity, both of which contribute to the blood pressure responsiveness. Delarue et al. (10) reported that the circulating plasma catecholamine response was blunted during mental stress after 1 mo of fish oil supplementation. More specifically, the attenuation was observed with epinephrine but not norepinephrine. It is well documented that mental stress elicits simultaneous increases of epinephrine and norepinephrine (14), but evidence also exists to suggest that increases in epinephrine during mental stress are more dramatic than the increases in norepinephrine (11, 27). It is important to note three things. First, plasma catecholamine responses do not always reflect MSNA responsiveness (17). Moreover, there are several factors that influence plasma catecholamine levels, including rates of secretion, clearance, and reuptake (18). Second, in contrast to the catecholamine responses, MSNA responses to mental stress are a bit more variable. Specifically, mental stress can elicit increases, decreases, or no change in MSNA (7), and it has been suggested that this "responsiveness" may be important in future development of cardiovascular disease (36). Much like the cardiovascular reactivity hypothesis, it remains unclear if this sympathetic neural reactivity is causal, predictive, or neither with regards to the pathogenesis of cardiovascular disease. Third, the study by Delarue et al. (10) did not include a placebo control. Collectively, there was a strong rationale to examine the influence of fish oil on MSNA reactivity to mental stress.

Consistent with our hypothesis, fish oil reduced total MSNA reactivity to mental stress. Contrary to our hypothesis, fish oil did

not alter MSNA burst frequency or burst incidence reactivity to mental stress. Similar to the divergent impact of fish oil on HR and MAP reactivity, it is not unheard of for burst frequency and total MSNA to respond differently to various interventions. In fact, this divergent sympathetic neural response may be an important distinction in understanding potential mechanisms underlying the effect of fish oil on the sympathetic nervous system during stress. Specifically, it has been suggested that sympathetic burst activity and total MSNA operate off of two separate central nervous system (CNS) inputs (26). The generation of a burst is suggested to be controlled through a gated (i.e., on/off) mechanism, whereas the total MSNA appears to operate via graded input. Whereas this gated mechanism may operate off of one CNS input, the graded mechanism may respond to multiple CNS inputs (25, 26). Keller et al. (25) demonstrated that whole body heating increased burst activity (i.e., via the gating mechanism), but did not affect the total MSNA response, supporting the theory presented by Kienbaum et al. (26) that these two mechanisms of muscle sympathetic nerve activity operate on two separate CNS inputs. Accordingly, one reasonable interpretation of the present data (i.e., blunted total MSNA reactivity to mental stress without a concomitant attenuation of burst frequency reactivity) is that fish oil might influence sympathetic neural "recruitment" more so than a sympathetic "gating."

Finally, improved vascular function has been proposed as a potential mechanism for the cardiovascular benefits associated with fish oil. In the present study we examined limb vascular responsiveness to mental stress. Because prehypertensive and hypertensive adults demonstrate blunted forearm vasodilation during mental stress compared with normotensive adults (6, 39, 41), we hypothesized that fish oil would augment forearm vasodilatory responses to mental stress. Our data demonstrate that fish oil did not alter the forearm vascular reactivity to mental stress (i.e., similar vasodilation). Moreover, fish oil actually appeared to blunt, not augment, calf vascular conductance during mental stress, a finding that was contrary to our initial hypothesis. Although somewhat perplexing, we offer the following potential explanation. The study by Delarue et al. (10) demonstrated increases in epinephrine. Epinephrine has been identified as a key contributor to the limb vasodilatory response of mental stress and may play an important role in nitric oxide-mediated vasodilation during mental stress (22). Thus, if the epinephrine response is indeed blunted as reported by Delarue et al. (10), perhaps this contributed to a blunted calf vasodilation. On the other hand, why would this not have also impacted the forearm vasodilatory response to mental stress? We are not entirely clear why fish oil elicited different limb vasodilatory responses to mental stress (i.e., forearm vs. calf), and this may warrant future investigation. To our knowledge, this is the first study to report limb vascular reactivity to mental stress after fish oil supplementation.

We acknowledge several limitations of the present work. First, this study was restricted to 8 wk of fish oil supplementation. Longer duration (i.e., chronic) fish oil supplementation and/or fish oil through natural diet may elicit more robust neural and cardiovascular changes. Second, the present study did not measure the amount of omega-3 fatty acid incorporated into plasma or red blood cells. It is possible that variable incorporation rates of omega-3 fatty acid could have influenced the treatment response in our study. Third, the present study was limited to younger individuals. Future work might examine older subjects and/or other populations with higher neural cardiovascular reactivity to mental stress. Finally, we do not have any measurements of stroke volume, thus limiting our interpretation regarding differences between the HR and MAP reactivity. Dyson et al. (12) has recently reported discrepancies of estimates of stroke volume between finometer ModelFlow and pulsed Doppler ultrasound of the ascending aorta during acute changes of TPR, and mental stress has been reported to elicit acute changes in TPR. Importantly, validation studies with the ModelFlow have primarily focused on changes during orthostasis; to our knowledge, there are no validation studies during mental stress. Given the recent findings of Dyson et al. (12) and the lack of direct ModelFlow validation studies during mental stress, we are not comfortable using the ModelFlow to estimate stroke volume reactivity in the present study.

Perspectives and Significance

The present study provides new mechanistic insight regarding the influence of fish oil supplementation on neural cardiovascular reactivity. Specifically, we report that that 8 wk of fish oil supplementation significantly attenuates both HR and total MSNA reactivity to mental stress. Overall, the data support and extend the growing evidence that fish oil may have positive health benefits regarding neural cardiovascular control in humans and suggest important physiological interactions between fish oil and psychological stress that may contribute to disease etiology. Future studies might examine the influence of longer duration (i.e., chronic) fish oil supplementation on neural cardiovascular reactivity to mental stress, with a particular focus on aged and/or diseased populations.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: J.R.C. and M.J.J. conception and design of research; J.R.C., C.E.S., and H.Y. performed experiments; J.R.C., C.E.S., and H.Y. analyzed data; J.R.C., C.E.S., H.Y., and M.J.J. interpreted results of experiments; J.R.C. and C.E.S. drafted manuscript; J.R.C., C.E.S., H.Y., and M.J.J. edited and revised manuscript; J.R.C., C.E.S., H.Y., and M.J.J. approved final version of manuscript; H.Y. prepared figures.

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