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Omega 3's Found to Have Positive Effects on Blood Flow and Blood Circulation

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Effects of Dietary Omega-3 Polyunsaturated Fatty Acids on the Skeletal-Muscle Blood-Flow Response to Exercise in Rats

Charles L. Stebbins, Lauren E. Hammel, Benjamin J. Marshal, Espen E. Spangenberg, and Timothy I. Musch

The polyunsaturated fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) affect vascular relaxation and involve factors (e.g., nitric oxide) that contribute to exercise-induced increases in skeletal-muscle blood flow (Q). The authors investigated whether DHA and EPA supplementation augments skeletal-muscle Q and vascular conductance (VC) and attenuates renal and splanchnic Q and VC in exercising rats. Rats were fed a diet of 5% lipids by weight, of which 20% was DHA and 30% EPA (PUFA group, $n = 9$), or 5% safflower oil (SO group, $n = 8$) for 6 wk. Heart rate (HR), blood pressure (MAP), and hind-limb, renal, and splanchnic Q were measured at rest and during moderate treadmill running. MAP, HR, and renal and splanchnic Q and VC were similar between the 2 groups at rest and during exercise. In the PUFA group, Q (158 ± 27 vs. 128 ± 28 ml · min^{-1} · 100 g^{-1}) and VC (1.16 ± 0.21 vs. 0.92 ± 0.23 ml · min^{-1} · 100 g^{-1} · mm Hg^{-1}) were greater in the exercising hind-limb muscle. Q and VC were also higher in 8 of 28 and 11 of 28 muscles and muscle parts, respectively. These increases were positively correlated to the percent sum of Types I and IIa fibers. Results suggest that DHA+EPA (a) enhances Q and VC in active skeletal muscle (especially Type I and IIa fibers) and that the increase in Q is due to an increase in cardiac output secondary to increases in VC and (b) has no apparent influence on vasoconstriction in renal and splanchnic tissue.

Keywords*:* docosahexaenoic acid, eicosapentaenoic acid, vascular conductance, heart rate, blood pressure, treadmill exercise

Treatment with the omega-3 polyunsaturated fatty acids (PUFAs) docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) has effects that may enhance skeletal-muscle blood flow during dynamic exercise. For example, 6 weeks of dietary supplementation with DHA and EPA augments increases in diameter and flow in conduit vessels (i.e., brachial arteries) supplying blood to human forearm muscles during intermittent static contraction (Walser, Giordano, & Stebbins, 2006). However, results of that study did not determine how these PUFA-induced increases in conduit-vessel diameter and flow affect distribution of blood flow to skeletal muscle during whole-body exercise. The capability of DHA and/or EPA to elevate blood flow in exercising muscle might depend on muscle-fiber type. A previous study of rats (Totland et al., 2000) reported that chronic feeding with EPA increases mitochondrial growth in Type I, highly oxidative, but not Type II, low oxidative but highly glycolytic fibers of skeletal muscle. Thus, this PUFA may also have differential vascular effects within and between muscles composed of these two distinct muscle-fiber types.

Dietary supplementation with DHA and EPA may also modify sympathetically induced distribution of cardiac output during exercise, which is characterized by reductions in blood flow and conductance in the renal and splanchnic circulation (e.g., stomach and small intestine; Rowell, 1993). On one hand, treatment with these fatty acids has been reported to enhance muscle sympathetic nerve activity to physiological stressors, including static exercise (Monahan, Wilson, & Ray, 2004). On the other, DHA and EPA have been shown to reduce levels of norepinephrine in conditions such as mental stress (Sawazaki, Hamazaki, Yazawa, & Kobayashi, 1999) and diabetes (Nishimura et al., 2000). Thus, it is not clear what, if any, effects DHA and EPA may have on blood flow and conductance in the splanchnic circulation during whole-body dynamic exercise. In contracting skeletal muscle, it may be that these PUFAs have local vasodilatory effects (e.g., enhanced endothelial function in response to shear stress) that predominate over their potential to enhance sympathetically induced vasoconstriction, while vasoconstriction may predominate in renal and splanchnic circulation.

We tested the hypothesis that dietary supplementation with DHA+EPA modifies the distribution of cardiac output during dynamic exercise and is characterized by augmentations in blood flow and vascular conductance in

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the contracting hind-limb muscles or muscle parts based on their oxidative capacity as estimated by their fiber-type composition (i.e., the cumulative percentage of Type I and Type IIa fibers). We also hypothesized that the increases in blood flow and conductance in skeletal muscle would be associated with reductions in these variables in nonactive tissue (i.e., kidneys, stomach, and small intestine).

Methods

Animal Selection and Care

A total of 17 adult male Sprague-Dawley rats were studied. They were maintained on a 12-hr–12-hr light–dark cycle and received food and water ad libitum. All experiments were approved under the guidelines established by the institutional animal care and use committees of both UC-Davis and Kansas State University and conducted under the guiding principles of the American Physiological Society and the National Institutes of Health.

Dietary Supplementation

Two groups of rats were studied. Both groups were fed the following diet (expressed as grams/kg diet): casein 225, cornstarch 446, sucrose 223, cellulose 31, DL-methionine 1, standard mineral mix 14, standard vitamin mix 10, oil 50, and butylhydroquinone 0.08. Both diets also consisted of 5% oil. In the control group, this was safflower oil (SO, $n = 8$), which has been shown to have no chronic effects on brachial-artery blood flow and conductance, blood pressure, or cardiac output during exercise (Walser et al., 2006). In the experimental group (PUFA, $n = 9$), menhaden oil, containing ~20% DHA and ~30% EPA, was added to the diet. The two diets were isocaloric, and they were followed for 6 weeks. A similar diet containing these levels of DHA and EPA has been reported to improve vascular function in rat aorta (López et al., 2004).

Treadmill Acclimatization

Rats in the PUFA and SO groups were familiarized with running on a motor-driven treadmill before initiation of the exercise protocols. They were placed into a lane on a treadmill belt where they were acclimated to exercising on the treadmill for 1–2 weeks. Exercise sessions of ~5 min were conducted at treadmill speeds ranging from 15 to 20 m/min up a 10% grade.

Surgical Procedures

Rats were initially anesthetized with a gas mixture of 5% isoflurane and 95% oxygen and then maintained on a 3% isoflurane and 97% oxygen mixture during surgical instrumentation. The right carotid and caudal (tail) arteries were isolated and cannulated with polyethylene catheters (PE-10 connected to PE-50, Intra-Medic polyethylene tubing, Clay Adams, Sparks, MD). The carotid-artery catheter was advanced to 2–3 mm rostral of the aortic valve. The caudal-artery catheter was advanced toward the bifurcation of the descending aorta. Both catheters were then tunneled subcutaneously to the dorsal aspect of the thorax and exteriorized through a small puncture wound in the skin. Subsequently, anesthesia was terminated, and a ≥2-hr period was provided for recovery. This period of recovery from gas anesthesia has been shown to be in a range that allows hemodynamic, arterial blood-gas, and acid-base levels to stabilize (Flaim et al., 1984).

Experimental Protocols

After the recovery period, rats were placed on the treadmill. Fifteen minutes later, the caudal-artery catheter was attached to a 1-ml plastic syringe connected to a Harvard infusion/withdrawal pump (Model 907). The carotid-artery catheter was connected to a pressure transducer to measure heart rate and blood pressure. Exercise was then initiated at a speed of 20 m/min up a 10% grade (~65% of VO_{2max}; Musch, Bruno, Bradford, Vayonis, & Moore, 1988). After ~3 min of exercise, ~0.5×10^6 radio-labeled microspheres (^{46}Sc or ^{85}Sr, 15 microns in diameter, Perkin-Elmer Life Sciences Inc., Boston, MA) were injected into the aortic arch (via the carotid-artery catheter) to determine blood flow to the kidneys, stomach, small intestine, and skeletal muscle of the hind limb. Simultaneously, blood was withdrawn from the caudal-artery catheter at a rate of 0.25 ml/min. Approximately 30 s after infusion of the microspheres, blood withdrawal and exercise were stopped, and the rats were allowed to recover for ~60 min. Subsequently, heart-rate and blood-pressure measurements were repeated, and the procedure for infusing microspheres was repeated to determine regional blood flow under postexercise resting conditions. This approach to measuring resting blood flow minimizes the potential for blood loss to affect measurements during exercise and circumvents potential preexercise anticipatory effects on blood flow (Armstrong, Hayes, & Delp, 1989).

After blood-flow determinations were completed, rats were removed from the treadmill and anesthetized with pentobarbital (40 mg/kg i.a.). They were then euthanized with an overdose of pentobarbital anesthesia (100 mg/kg i.a.). Next, the thorax was opened and placement of the carotid-artery catheter into the aortic arch was confirmed by anatomical dissection. The kidneys, representative organs of the splanchnic circulation (small intestine and stomach), and muscles and muscle parts of both hind limbs (listed in Tables 1 and 2) were removed, weighed, and placed in counting vials. The radioactivity of each tissue was determined by a gamma-scintillation counter (Packard Auto Gamma spectrometer, Model 5230, Downers Grove, IL). Taking into account the cross-talk fraction between isotopes, blood flow to each tissue was determined using the reference sample method and expressed in $ml \cdot min^{-1} \cdot 100\ g^{-1}$ tissue (Musch &Terrell, 1992). Adequate mixing of the microspheres was confirmed if there was <15% difference in right- and left-kidney blood flows. Blood-flow measurements were also normalized to mean arterial pressure and expressed as vascular conductance ($ml \cdot min^{-1} \cdot 100\ g^{-1} \cdot mm\ Hg^{-1}$).

Table 1 Resting Blood Flow and Conductance in Hind-Limb Muscles of Rats Supplemented With Safflower Oil or the Polyunsaturated Fatty Acids (PUFAs) DHA and EPA, *M* ± *SD*

	Blood Flow ($ml \cdot min^{-1} \cdot 100\ g^{-1}$)		Conductance ($ml \cdot min^{-1} \cdot 100\ g^{-1} \cdot mm\ Hg^{-1}$)	
	Safflower oil	PUFAs	Safflower oil	PUFAs
Ankle Extensors				
Soleus	120 ± 54	94 ± 72	0.86 ± 0.40	0.68 ± 0.54
Plantaris	12 ± 7	21 ± 27	0.09 ± 0.06	0.15 ± 0.21
Gastrocnemius				
red	24 ± 8	37 ± 48	0.17 ± 0.08	0.27 ± 0.36
white	17 ± 11	17 ± 9	0.12 ± 0.08	0.12 ± 0.06
middle	12 ± 6	17 ± 21	0.08 ± 0.06	0.12 ± 0.15
Tibialis posterior	29 ± 23	23 ± 21	0.21 ± 0.17	0.17 ± 0.15
Flexor digitorum longus	49 ± 20	51 ± 21	0.36 ± 0.37	0.37 ± 0.18
Flexor hallicus longus	13 ± 9	20 ± 24	0.10 ± 0.06	0.15 ± 0.18
Ankle Flexors				
Tibialis anterior				
red	50 ± 40	59 ± 58	0.36 ± 0.28	0.43 ± 0.14
white	23 ± 14	27 ± 27	0.16 ± 0.11	0.20 ± 0.21
Extensor digitorum longus	22 ± 14	32 ± 39	0.16 ± 0.11	0.24 ± 0.30
Peroneals	16 ± 11	23 ± 24	0.12 ± 0.08	0.17 ± 0.18
Knee Extensors				
Vastus intermedius	81 ± 51	93 ± 6	0.58 ± 0.37	0.69 ± 0.60
Vastus medialis	13 ± 6	26 ± 30	0.09 ± 0.06	0.20 ± 0.30
Vastus lateralis				
red	85 ± 51	89 ± 105	0.61 ± 0.40	0.65 ± 0.78
white	16 ± 14	18 ± 21	0.11 ± 0.08	0.13 ± 0.15
middle	25 ± 14	27 ± 33	0.18 ± 0.11	0.20 ± 0.27
Rectus femoris				
red	19 ± 14	49 ± 54	0.14 ± 0.11	0.36 ± 0.42
white	16 ± 11	24 ± 24	0.11 ± 0.08	0.18 ± 0.18
Knee Flexors				
Biceps femoris				
anterior	10 ± 6	15 ± 21	0.57 ± 0.17	0.65 ± 0.24
posterior	12 ± 8	13 ± 15	0.08 ± 0.06	0.10 ± 0.12
Semitendinosus	11 ± 6	14 ± 12	0.08 ± 0.03	0.10 ± 0.09
Semimembranosus				
red	15 ± 9	19 ± 24	0.11 ± 0.08	0.14 ± 0.18
white	12 ± 6	15 ± 15	0.08 ± 0.03	0.11 ± 0.12
Thigh Adductors				
Adductor longus	123 ± 57	143 ± 60	0.87 ± 0.42	1.05 ± 0.45
Adductor magnus and brevis	14 ± 6	21 ± 24	0.10 ± 0.06	0.16 ± 0.18
Gracilis	14 ± 6	15 ± 9	0.10 ± 0.06	0.11 ± 0.09
Pectineus	35 ± 31	38 ± 45	0.26 ± 0.23	0.28 ± 0.36

Note. DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid.

Table 2 Blood Flow and Conductance During Submaximal Treadmill Exercise in Hind-Limb Muscles of Rats Supplemented With Safflower Oil or the Polyunsaturated Fatty Acids (PUFAs) DHA and EPA, *M* ± *SD*

	Blood Flow ($ml \cdot min^{-1} \cdot 100\ g^{-1}$)		Conductance ($ml \cdot min^{-1} \cdot 100\ g^{-1} \cdot mm\ Hg^{-1}$)	
	Safflower oil	PUFAs	Safflower oil	PUFAs
Ankle Extensors				
Soleus	250 ± 96	330 ± 86*	1.79 ± 0.71	2.43 ± 0.66*
Plantaris	222 ± 88	263 ± 72	1.58 ± 0.62	1.92 ± 0.57
Gastrocnemius				
red	453 ± 190	515 ± 162	3.22 ± 0.37	3.79 ± 1.23
white	45 ± 20	70 ± 36*	0.33 ± 0.17	0.51 ± 0.24*
middle	184 ± 71	232 ± 60	1.31 ± 0.54	1.71 ± 0.45
Tibialis posterior	198 ± 65	263 ± 99	1.42 ± 0.48	1.92 ± 0.69
Flexor digitorum longus	83 ± 51	122 ± 36	0.59 ± 0.37	0.89 ± 0.27*
Flexor hallicus longus	116 ± 45	158 ± 45*	0.83 ± 0.34	1.16 ± 0.33*
Ankle Flexors				
Tibialis anterior				
red	289 ± 91	373 ± 114	2.07 ± 0.74	2.75 ± 0.90
white	110 ± 51	124 ± 39	0.79 ± 0.40	0.91 ± 0.30
Extensor digitorum longus	76 ± 40	92 ± 33	0.55 ± 0.31	0.67 ± 0.24
Peroneals	109 ± 45	141 ± 48	0.78 ± 0.34	1.04 ± 0.39
Knee Extensors				
Vastus intermedius	418 ± 147	500 ± 141	2.99 ± 1.08	3.67 ± 1.05
Vastus medialis	152 ± 48	225 ± 111*	1.09 ± 0.37	1.64 ± 0.72*
Vastus lateralis				
red	407 ± 150	414 ± 132	2.91 ± 1.10	3.04 ± 0.99
white	92 ± 119	85 ± 48	0.63 ± 0.76	0.62 ± 0.33
middle	242 ± 82	251 ± 72	1.73 ± 0.62	1.84 ± 0.51
Rectus femoris				
red	277 ± 99	360 ± 90*	1.99 ± 0.76	2.65 ± 0.66*
white	131 ± 40	171 ± 48*	0.94 ± 0.28	1.26 ± 0.36*
Knee Flexors				
Biceps femoris				
anterior	76 ± 28	97 ± 39	0.61 ± 0.08	0.71 ± 0.27
posterior	94 ± 28	117 ± 30	0.67 ± 0.25	0.86 ± 0.24
Semitendinosus	46 ± 14	59 ± 27	0.33 ± 0.11	0.43 ± 0.21
Semimembranosus				
red	175 ± 54	215 ± 66	1.25 ± 0.42	1.58 ± 0.48*
white	48 ± 14	74 ± 27*	0.34 ± 0.11	0.54 ± 0.18*
Thigh Adductors				
Adductor longus	247 ± 164	450 ± 222*	1.76 ± 1.16	3.33 ± 1.74*
Adductor magnus and brevis	102 ± 28	126 ± 42	0.73 ± 0.23	0.93 ± 0.33
Gracilis	38 ± 17	69 ± 51	0.27 ± 0.11	0.51 ± 0.36*
Pectineus	36 ± 23	51 ± 39	0.26 ± 0.17	0.38 ± 0.27

Note. DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid.

*$p \leq .05$ vs. safflower oil.

Data Analysis

Heart rate, mean arterial blood pressure, skeletal-muscle blood flow, and vascular conductance were compared between the two groups using an unpaired Student's *t* test. Regression analysis was performed to determine whether differences (i.e., increases) in absolute blood flow and vascular conductance to the individual hind-limb muscles or muscle parts found between the SO and PUFA rats during exercise were correlated with their estimated fiber-type composition (Delp & Duan, 1996). All values are expressed as $M \pm SD$. Statistical significance was set at $p < .05$.

Results

There were no differences in body weight between the SO and PUFA rats either before (SO 481 ± 23 g, PUFA 486 ± 45 g) or after 6 weeks of supplementation (SO 534 ± 37 g, PUFA 551 ± 45 g).

PUFAs' Effects on Heart Rate and Mean Arterial Pressure

Heart rate and mean arterial pressure were not different between the SO and PUFA rats at rest or during exercise (Table 3).

PUFAs' Effects on Muscle Blood Flow and Conductance

Resting blood flow and conductance to the total hind-limb musculature were similar between the SO and PUFA rats (Figure 1, upper and lower left panels) and in the 28 hind-limb muscles or muscle parts examined (Table 1). During exercise, the PUFA group exhibited a 23% greater blood flow to the total hind-limb musculature (PUFA, 158 ± 27 ml · min^{-1} · 100 g^{-1}; SO, 128 ± 28 ml · min^{-1} · 100 g^{-1}; Figure 1, upper right panel). This reflected, in part, a significantly higher blood flow found in 8 of the 28 hind-limb muscles or muscle parts than in their SO counterparts (Table 2 and Figure 2, upper panel). In addition, conductance in the PUFA animals was 26% greater to the hind-limb musculature during exercise (PUFAs 1.16 ± 0.21 ml · min^{-1} · 100 g^{-1} · mm Hg^{-1}; SO 0.92 ± 0.23 ml · min^{-1} · 100 g^{-1} · mm Hg^{-1}; Figure 1, lower right hand panel) and is reflected, in part, by significantly greater conductance in 11 of the 28 hind-limb muscles or muscle parts examined (Table 2 and Figure 2, lower panel).

Shown in Figure 3 are the relationships during exercise between estimated fiber-type distribution and increases in absolute blood flow and conductance found in the hind-limb muscles or muscles parts when the PUFA and SO groups were compared. Augmentations in blood flow (upper panel) and conductance (lower panel) observed in the PUFA rats were significantly correlated with the cumulative percentage of Type I and Type IIa fibers found in each of the hind-limb muscles or muscle parts.

Table 3 Heart Rate (HR) and Mean Arterial Blood Pressure (MAP) Measured at Rest and During Exercise in Rats Fed a Diet Supplemented With Safflower Oil or the Polyunsaturated Fatty Acids (PUFAs) DHA and EPA, *M* ± *SD*

	HR (beats/min)	MAP (mm Hg)
Safflower oil		
rest	417 ± 14	140 ± 8
exercise	500 ± 17*	141 ± 8
PUFAs		
rest	412 ± 33	137 ± 9
exercise	492 ± 21*	136 ± 3

Note. DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid.

*$p \leq .05$ vs. rest.

PUFAs' Effects on Renal, Stomach, and Small Intestine Blood Flow and Conductance

Renal, stomach, and small intestine blood flows in the PUFA rats were not different from those in the SO animals at rest or during exercise (Figure 4, upper panels). Conductance in the kidney, stomach, and small intestine at rest and during exercise also was not different between the PUFA and SO groups (Figure 4, lower panels).

Discussion

Results of this study indicate that dietary supplementation with the PUFAs DHA and EPA is capable of augmenting contraction-induced increases in skeletal-muscle blood flow and conductance and resulting in strong correlations between changes in these two variables and the percent sum of Type I and IIa muscle fibers (i.e., oxidative fibers) observed in the individual muscles or muscle parts. In addition, these augmentations in blood flow and conductance occurred in the absence of any changes in perfusion pressure (i.e., mean arterial pressures) or heart rate. These responses indicate that supplementation with DHA+EPA may effectively alter vasoreactivity in the vasculature of skeletal muscle.

We previously found that supplementation with DHA+EPA enhances blood flow and conductance in conduit arteries (i.e., brachial arteries) that direct blood flow to skeletal muscle in the human forearm (Walser et al., 2006). Our current results support these findings and provide new information suggesting that these PUFAs also increase blood flow in resistance vessels that directly supply the active skeletal muscle and that this effect is mediated by increases in vascular conductance. This finding is important because increases in conduit-artery blood flow alone would be expected to have a limited effect on skeletal-muscle blood flow in the absence of concomitant

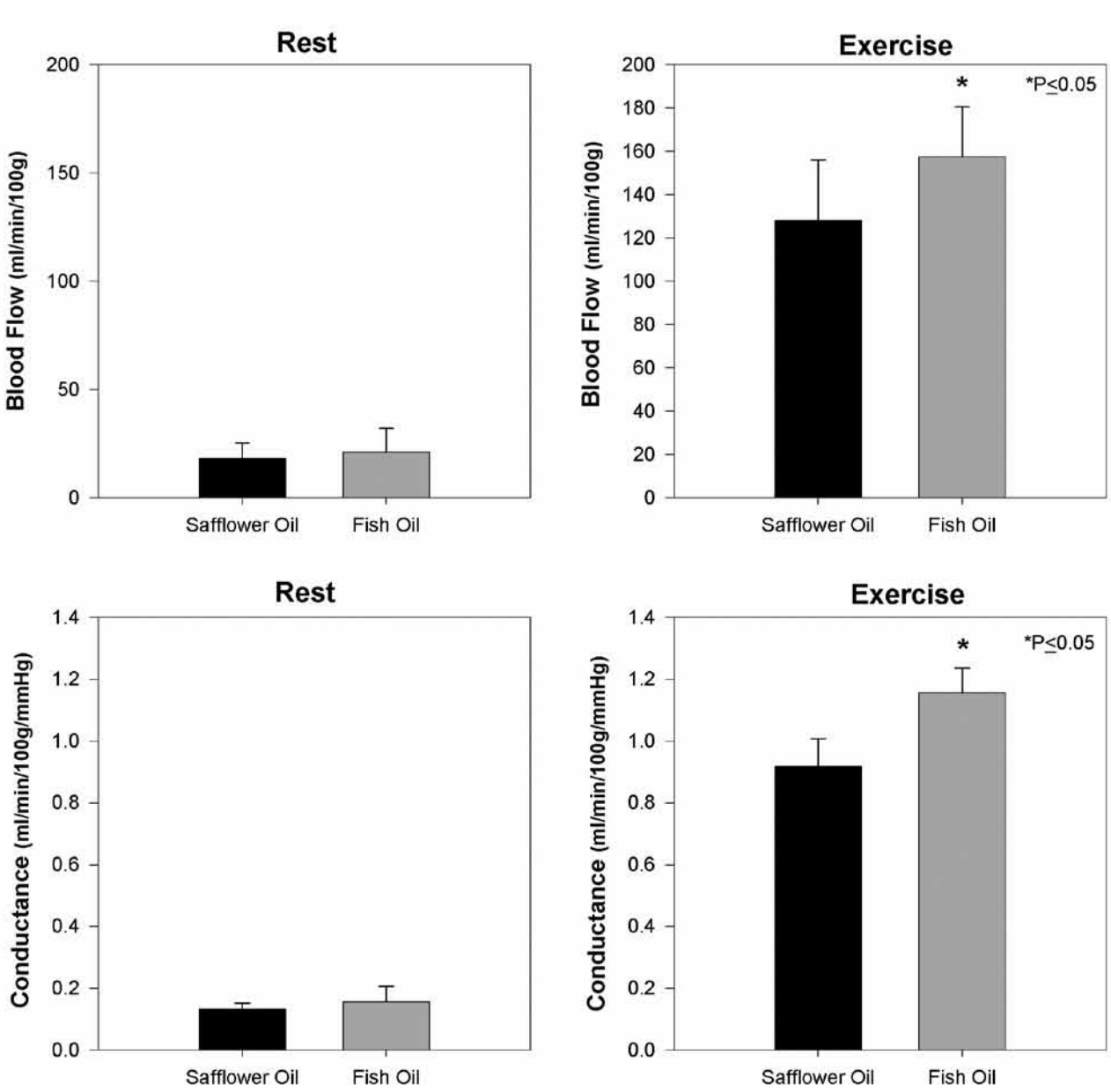

Figure 1 — Total hind-limb-muscle blood flow (upper left and right panels) and conductance (lower left and right panels) measured at rest and during submaximal exercise in rats supplemented with dietary safflower oil or the polyunsaturated fatty acids docosahexaenoic acid and eicosapentaenoic acid, $M \pm SE$. $^*p \leq .05$ vs. safflower oil.

increases in conductance in resistance arteries (e.g., feed arteries and arterioles).

Our results suggest that the DHA+EPA-induced increases in blood flow and conductance during exercise were dependent on the percentage of oxidative fibers (i.e., Type I and Type IIa fibers) found in each individual muscle or muscle part examined. These findings are consistent with those of previous studies suggesting that muscles of high oxidative capacity (i.e., predominantly composed of Type I and Type IIa fibers) demonstrate endothelium-mediated vasodilation via the nitric oxide (NO) pathway (McAllister, 2003; Delp, Colleran, Wilkerson, McCurdy, & Muller-Delp, 2000; Wunsch, Muller-Delp, & Delp, 2000) and that PUFAs can enhance NO production (Li et al., 2007). However, we cannot dismiss the possibility that our results were influenced by our exercise regimen and the specific pattern of muscle recruitment required to achieve the necessary work intensity. In this vein, the treadmill speed we used likely recruited muscles composed mostly of Type I and Type IIa fibers; muscles with primarily Type IIb fibers would not have been recruited unless greater treadmill speeds were imposed (Armstrong & Laughlin, 1985; Laughlin & Armstrong, 1982). Thus, a higher workload may have caused greater PUFA-induced

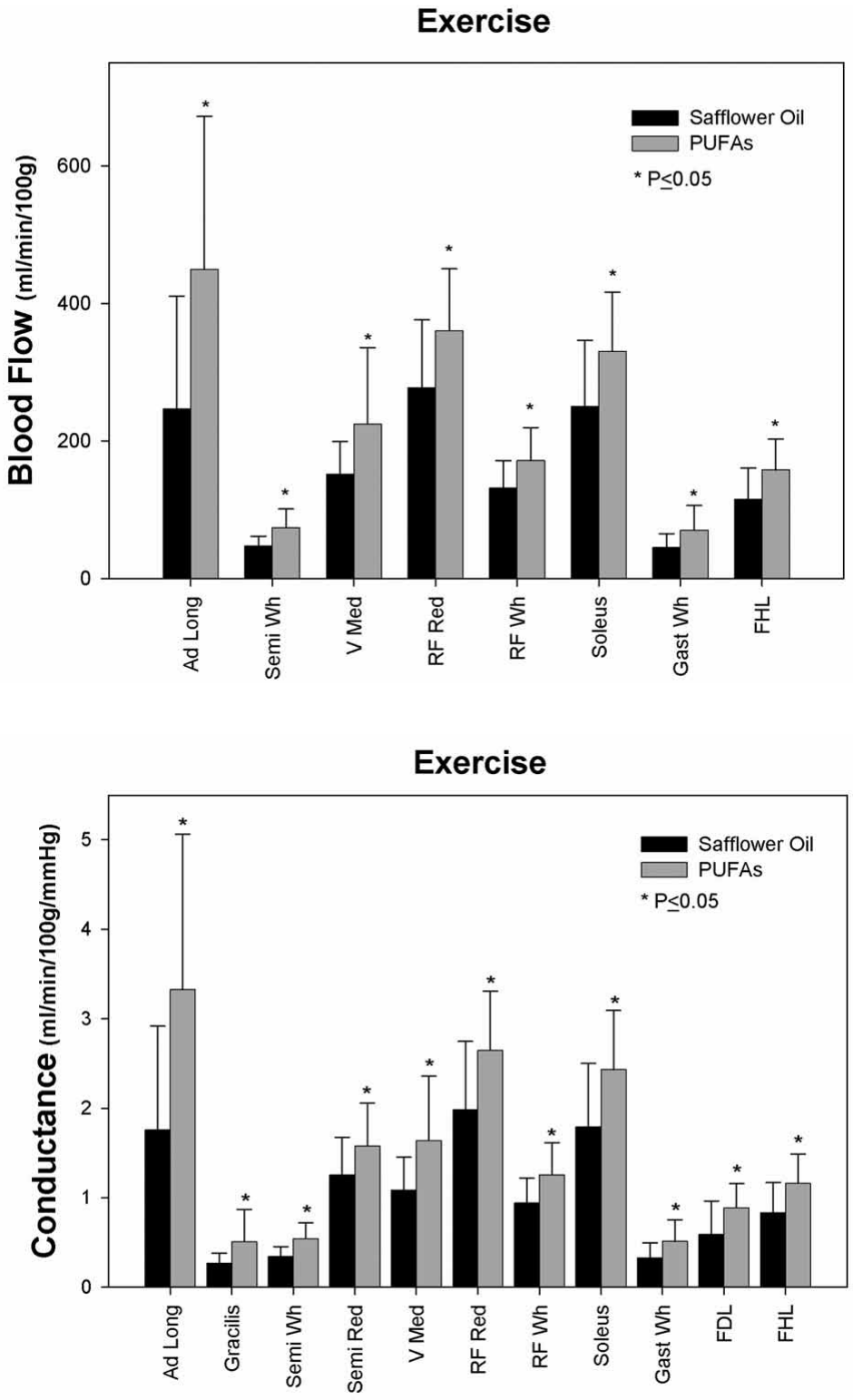

Figure 2 — Blood flow (upper panel) and conductance (lower panel) measured during submaximal exercise in specific locomotor muscles or muscle parts of the hind limb that were significantly greater after dietary supplementation with the polyunsaturated fatty acids (PUFAs) docosahexaenoic acid and eicosapentaenoic acid compared with safflower oil, $M \pm SE$. Ad Long = adductor longus, Semi Wh = white portion of the semimembranosus, Semi Red = red portion of the semimembranosus, V Med = vastus medialis, RF Red = red portion of the rectus femoris, RF Wh = white portion of the rectus femoris, Gast Wh = white portion of the gastrocnemius, FDL = flexor digitorum longus; FHL = flexor hallicus longus. $^*p \leq 0.05$ vs. safflower oil.

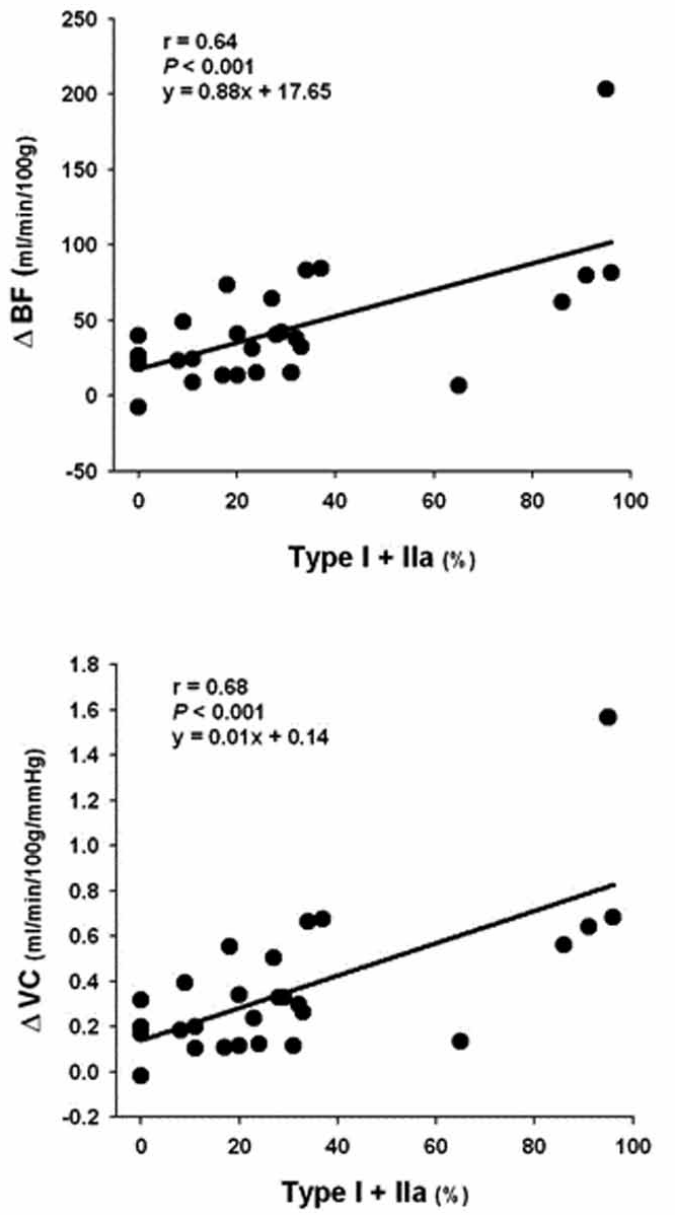

Figure 3 — Relationships during exercise between the percentage of Type I and IIa fibers in the individual locomotor muscles or muscle parts of the hind limb and changes in blood flow (BF) and (VC) conductance in the polyunsaturated fatty acids group compared with the safflower oil rats.

augmentations in blood flow and conductance in a larger number of muscles. However, this potential limitation does not detract from the beneficial effects on skeletal-muscle blood flow and conductance that we did find in response to PUFA supplementation.

The fact that blood pressure and heart rate were not different between the two groups raises a question concerning the source of the increased flow in the contracting muscle of the PUFA group. We believe it is because of a concomitant decrease in resistance (i.e., an increase in conductance) in the contracting muscles that we saw the demonstrated increases in blood flow. Our contention is supported by the results of a study in humans in which PUFA supplementation enhanced cardiac output in the absence of changes in heart rate and blood pressure (Walser & Stebbins, 2008). This increase was caused by an increase in stroke volume secondary to decreases in systemic vascular resistance.

The results did not confirm our second hypothesis, that DHA+EPA supplementation would reduce blood flow in renal and splanchnic circulation. Our rationale for this contention was based, indirectly, on the study of Monahan et al. (2004), whose treatment with DHA+EPA increased muscle sympathetic nerve activity in response

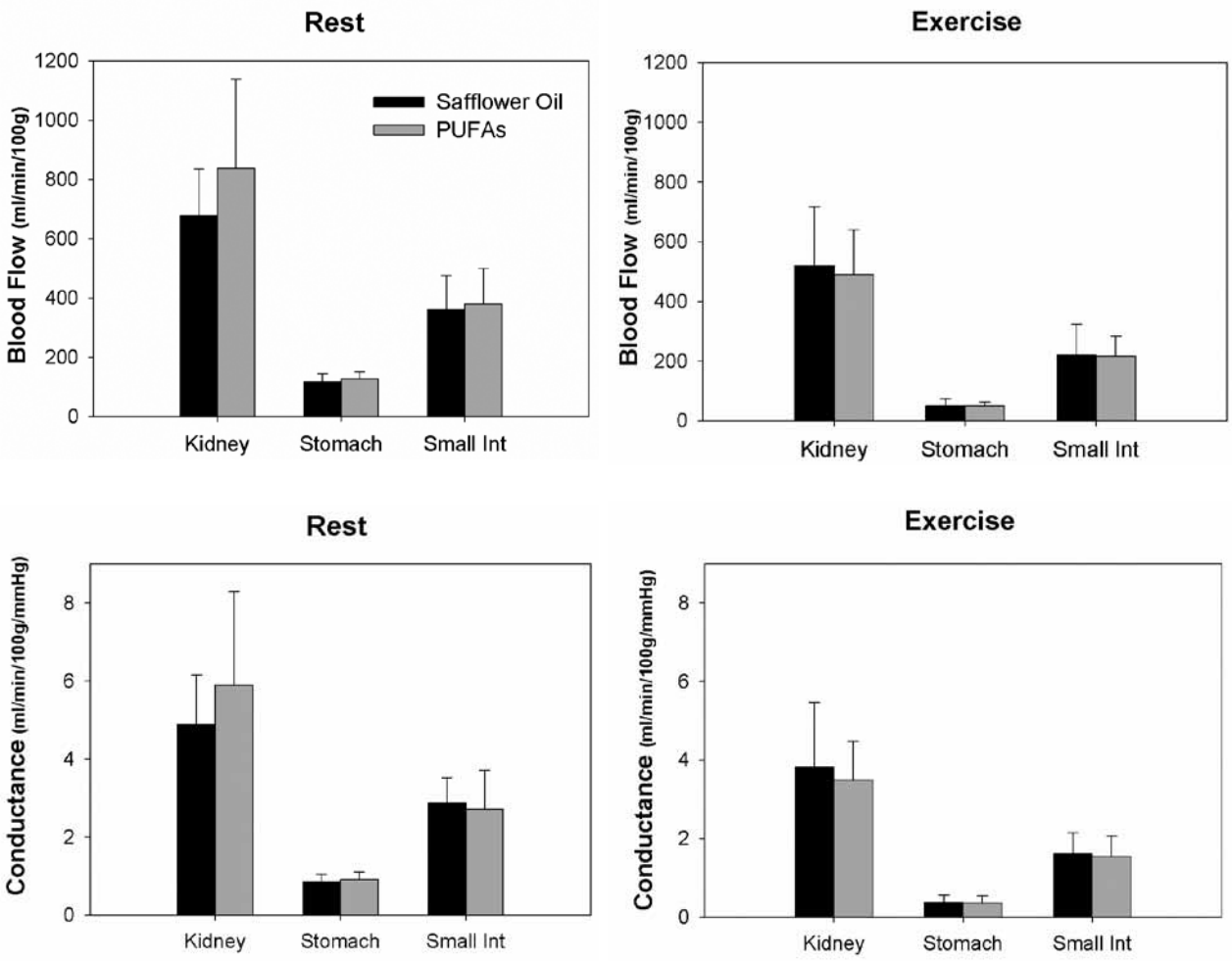

Figure 4 — Blood flow (upper panel) and conductance (lower panel) at rest and during submaximal exercise in the kidney, stomach, and small intestine (Small Int) after dietary supplementation with the polyunsaturated fatty acids (PUFAs) docosahexaenoic acid and eicosapentaenoic acid compared with safflower oil. There were no significant differences between groups.

to forearm exercise. Because exercise increases sympathetic outflow to the skeletal-muscle, renal, and splanchnic circulations (DiCarlo, Chen, & Collins, 1996; Koba, Xing, Sinoway, & Li, 2007; Miki, Kosho, & Hayashida, 2002), we assumed that any DHA+EPA-induced increases in sympathetic outflow to skeletal muscle would be accompanied by concomitant increases to the vasculature of the kidney, stomach, and small intestine. However, compared with the SO group (placebo conditions), blood flow and conductance in these organs were not different during exercise. This observation suggests that either sympathetically evoked vasoconstriction was not altered or metabolic and/or endothelium-evoked vasodilation offset effects of any increase in sympathetic nerve activity. Because there is little evidence supporting local vasodilatory effects on splanchnic flow during exercise (Rowell, 1993), DHA+EPA likely had no real effect on sympathetically induced vasoconstriction in these organs.

Although our results from the renal and splanchnic vasculature suggest that sympathetic effects on blood flow were not altered by PUFA treatment, we cannot rule out the possibility that local inhibitory effects contributed to the increased blood flow and conductance seen in the contracting muscle. In this regard, DHA+EPA can blunt norepinephrine-induced vasoconstriction in skeletal muscle (Chin, Gust, Nestel, & Dart, 1993). This effect might be a result of the ability of these PUFAs to enhance the bioavailability of NO (Li et al., 2007). Because NO can enhance functional sympatholysis in the vasculature of exercising skeletal muscle via inhibitory effects on α1-adrenergic receptors (Buckwalter, Taylor, Hamann, & Clifford, 2004), increases in its production might enhance this effect.

Potential Mechanisms Underlying PUFAs' Effects

It appears that the effects of PUFA treatment on active skeletal-muscle blood flow were primarily expressed peripherally. One underlying mechanism may involve

release of NO from the vascular endothelium. Shear stress, which is increased in conduit vessels and the vasculature of skeletal muscle during exercise (Niebauer & Cook, 1996), enhances endothelial production of NO (Rubanyi, Romero, & Vanhoutte, 1986). In rats, NO has been shown to contribute to increases in skeletal-muscle blood flow during exercise (especially in Type I and IIa fibers; Copp, Hirai, Hageman, Poole, & Musch, 2010; Hirai, Visneski, Kearns, Zelis, & Musch, 1994), and interstitial blockade of the primary enzyme responsible for its synthesis (NO synthase) can attenuate exercise hyperemia (Hickner, Fisher, Ehsani, & Khort, 1997). Because DHA+EPA can enhance NO bioavailability (Li et al., 2007), this response may lead to increases in active-muscle blood flow.Another possible factor underlying DHA+EPA effects on exercise-induced increases in skeletal-muscle blood flow is prostaglandins, substances that are released in response to exercise and that can contribute to the concomitant increase in skeletal-muscle blood flow (Copp et al., 2010; Duffy, New, Tran, Harper, & Meredith, 1999; Wilson & Kapoor, 1993). These substances are released, in part, from the vascular endothelium in response to elevations in shear stress (Cannon, 1984; Grabowski, Jaffe, & Weksler, 1985). Because PUFAs can facilitate endothelial release of vasodilator prostaglandins (Hishinuma, Yamazaki, and Mizugaki, 1999), this effect may contribute to flow-mediated vasodilation in contracting skeletal muscle.

During exercise, DHA+EPA supplementation also could affect muscle blood flow indirectly, via effects on muscle biology and/or metabolism. Metabolic effects of fish oils on skeletal muscle have been reported and include reductions in plasma glucose fluxes during exercise (Delarue, Labarthe, & Cohen, 2003) and increases in insulin-induced glucose transport (Sohal, Baracos, & Clandinin, 1992). These effects could potentially modulate muscle substrate metabolism and, in turn, alter metabolically induced dilation of arterioles and shear stress during exercise.

Limitations of the Study

A potential limitation of our study was our use of SO as a placebo oil. Although SO has not been shown to have effects on blood pressure, heart rate, or brachial-artery blood flow during exercise in humans (Walser et al., 2006), these variables have not been assessed in rats. Although we feel that such effects are unlikely, beneficial effects of a high-SO diet on cardiac morphology and function have been reported in hypertensive rats (compared with effects of a high-carbohydrate diet; Chicco et al., 2008). Consequently, we cannot eliminate the possibility that SO supplementation affected skeletal-muscle blood flow and conductance.

Perspectives

The relevance of our results relates to the capability of DHA+EPA-induced increases in blood flow and conductance to enhance oxygen delivery to contracting skeletal muscle. This effect may delay the onset of fatigue such that a given level of exercise can be maintained for a longer time period. Dietary supplementation with PUFAs may also represent an effective intervention for patients who exhibit exercise intolerance (e.g., heart failure or diabetes), because this condition is associated with endothelial dysfunction, reduced skeletal-muscle blood flow, and increased vascular resistance (Katz, 1999).

Conclusions

Results demonstrate that dietary supplementation with DHA+EPA augments skeletal-muscle blood flow in response to acute dynamic exercise and that this augmentation is related to the total percentage of Type I and IIa oxidative fibers in the individual muscles or muscle parts. In the hind limb, these changes were associated with an increase in overall blood flow. This response was a result of increases in conductance in resistance arterioles, not elevations in perfusion pressure. There were no changes in blood flow or conductance in the renal or splanchnic circulation, so it appears that these PUFAs had no effects on the vasculature of or sympathetic outflow to these organs. Findings suggest that DHA+EPA supplementation can enhance perfusion of contracting skeletal muscle without influencing the redistribution of splanchnic blood flow that occurs at the onset of exercise. We contend that the source of this elevation in skeletal-muscle blood flow is an increased cardiac output caused by a decrease in systemic vascular resistance that is concomitant with PUFA-induced increases in muscle conductance.

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