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Fish oil and oleic acid–rich oil feeding alter nucleoside uptake in human erythrocytes

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In the human erythrocyte, adenosine and other nucleosides are transported by facilitated diffusion via a single type of nitrobenzylthioinosine (NBMPR)-sensitive transporter. Erythrocytes contribute substantially to the uptake, metabolism, and release of adenosine metabolites in the circulation and thus, modulation of transporter activity could indirectly mediate responses to adenosine or its metabolites. We show here that supplementation of the human diet with oleic acid–rich oil or with fish oil containing high levels of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), results in decreases in nucleoside uptake across the erythrocyte plasma membrane. Oil feeding resulted in decreases in transport rates (pmol/10^7 cells/sec) for adenosine (1.65 ± 0.04 to 1.27 ± 0.02), thymidine (11.4 ± 0.3 to 9.4 ± 0.1), and uridine (8.3 ± 0.26 to 7.02 ± 0.09) on day 0 and day 42, respectively. Although changes in fatty acid composition were documented in the fish oil supplemented group, over the 6 week treatment period, changes in the oleic acid–rich oil supplemented group were not statistically significant. We suggest that fatty acid changes in membrane phospholipids may be responsible for the observed decreases in the rates of nucleoside transport in fish oil–fed subjects but that oleic acid supplementation may alter nucleoside uptake by another mechanism. Thus, in addition to having direct effects on platelet and neutrophil activity, oils or foods rich in oleic acid and/or omega-3 fatty acids may have indirect effects on the erythrocyte that potentiate the activity of adenosine on platelets and the vascular endothelium. (J. Nutr. Biochem. 6:438–444, 1995.)

Keywords: nucleoside; adenosine; erythrocyte; fish oil; oleic acid

Introduction

Although *de novo* synthesis of nucleosides is common in most tissues, nucleosides are provided via salvage pathways in several cell types (red blood cells, brain cells, and bone marrow) where *de novo* synthesis of purines is deficient.[1] Nucleosides, particularly adenosine, act as neuromodulators in brain.[2] ATP and adenosine hyperpolarize the presynaptic cell, inhibit neuronal firing, and inhibit transmitter release from cholinergic and adrenergic central neurons.[3] Very low levels of adenosine (<1 μM) or increased concentrations of ADP lead to platelet aggregation, while increased levels of adenosine (2- to 3-fold) result in significant inhibition of platelet aggregation.[4,5] Adenosine, probably through its action on A_1 and A_2 adenosine receptors, has critical effects on the cardiovascular system.[6,7] Even small changes in endogenous levels of adenosine can lead to dramatic changes in cardiovascular tone and cerebral blood flow[8] (high levels lead to vasodilation) with little or no effect on blood flow in skeletal muscle or in adipose tissue. Tissue levels of adenosine are in large part determined by intracellular and extracellular enzymatic degradation of adenosine nucleosides, by intracellular degradation of S-adenosylhomocysteine to adenosine and homocysteine, and by nucleoside transporter activity.[1,4] Studies utilizing nucleoside transporter inhibitors including dipyridamole, dilazep, and nitrobenzylthioinosine have confirmed the pivotal role adenosine transport plays in the antiplatelet and vasodilatory actions of these drugs.[5,9–11] Also, defects in nucleoside transport have been identified in diabetic animals and humans at risk of developing type I diabetes.[12,13]

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The dominant route for adenosine elimination is cellular uptake and degradation.[4,6,14,15] Since most nucleosides are hydrophillic and diffuse through the plasma membrane slowly, their uptake by cells depends on the presence of specialized transport proteins in the plasma membrane. Most cell types possess high-capacity nonconcentrative (equilibrative) nucleoside transport systems with broad permeant specificity and a few specialized cell types (e.g., intestinal epithelia, neurons and peritoneal macrophages) also possess Na^+-dependent concentrative systems.[16–21] Human erythrocytes possess a single type of nucleoside transporter which is sensitive ($K_i \sim 1$ nM) to the inhibitor nitrobenzylthioinosine (NBMPR).[22]

Epidemiologic studies have shown that populations whose diets are rich in omega-3 fatty acids (from fish and marine mammals) or vegetable oils rich in monounsaturated fatty acids (such as oleic acid as found in olive oil) have lower incidence of cardiovascular disease.[23–27] Intervention studies have concluded that supplementation with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) may delay the onset and progression of advanced atherosclerotic disease.[28] On the other hand, as Hornstra[29] has outlined, few intervention studies have compared fish oils with other dietary oils. Several studies have suggested that growth factor activity, transport of nutrients, or production of biological effector molecules can be modified by culturing cells or supplementing diets with polyunsaturated fatty acids (PUFAs).[28,30–35] Significant modification of the fatty acid composition of plasma membranes can be achieved through dietary intervention in whole animals[36,37] and even modest changes in membrane fatty acid composition (principally replacement of omega-6 fatty acids with omega-3 fatty acids) leads to altered membrane fluidity.[28] Studies focusing on the red cell in particular have concluded that maximum incorporation of DHA and EPA can occur within 6 weeks of feeding supplements.[38]

Since adenosine and its analogs play such important roles in the cardiovascular system and because erythrocyte transport and subsequent metabolism is a critical modulator of local levels of adenosine, we questioned whether the cardiovascular benefits of diets rich in oleic acid and long chain omega-3 fatty acids might not be partially mediated through changes in adenosine metabolism. Specifically we questioned whether dietary supplementation with fish or oleic acid–rich oils could alter the nucleoside uptake in the human erythrocyte and thus potentially alter adenosine mediated responses. Results of another component of this study have been reported elsewhere.[39]

Methods and materials

Subjects

Twenty healthy male volunteers (3 smokers) who had abstained from medication and maintained usual dietary habits for at least 2 weeks prior to the study's commencement were entered into the trial. We considered these individuals "free living normal subjects" and did not monitor their daily dietary intake except to ensure that they took the supplement and abstained from medication throughout the study period. Approval for the trial was granted by the Human Ethics Committee of the University of Guelph and the subjects were requested to sign informed consent forms. Subjects were randomly assigned into an oleic acid–rich oil or fish oil supplemented group. The supplements (provided by Ross Laboratories a division of Abbott Laboratories, Columbus, OH USA) were nutritionally complete (including protein [9.9 g], carbohydrate [19.7 g], vitamins, and minerals; patent pending) liquid formulations (250 ml each) containing 13.8 g of either oleic acid–rich oil (a mixture of safflower oil with a high oleic acid content and soy oils) or fish oil (sardine oil) providing a total energy intake of 995 kJ. Each serving containing fish oil provided 3.3 g of EPA (20:5n-3) and 1.2 g of DHA (22:6n-3), while each oleic acid–rich oil serving provided 6.3 g of oleic acid (18:1n-9). The fatty acid compositions of the supplements are given in *Table 1*. Compositions were determined by Lipid Analytical Laboratories (University of Guelph Research Park, Guelph, Ontario, Canada) using procedures similar to those described by Holub and Skeaff (1987)[44] with the modification that gas-liquid chromatography was used to separate methylated fatty acids. Formulations were taken each day at lunch time and subjects were requested to maintain their usual dietary habits for the entire experimental period (42 days). Subject characteristics are given in *Table 2*. None of these characteristics changed statistically over the experimental period. Some individuals were not analyzed on both days 0 and day 42 because of illness, medication use, or difficulty with sample preparation. Only individuals for which both day 0 and day 42 values were available were included in the various analyses.

Transport assay

Blood (280–300 ml) was obtained by anticubital venepuncture into siliconized evacuated bottles containing acid-citrate-dextrose as an anticoagulant[40] at day 0 (pretreatment) and day 42 (posttreatment). Platelets were removed by the method of Mustard et al.[41] and the red cell pellet (98% red cells) was washed four times at 22°C in 3 mM K_2HPO_4, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 144 mM NaCl, 20 mM Tris, pH 7.4, 10 mM glucose (transport buffer). Suspensions were counted in an electronic Coulter Counter (model ZM) and incubated with or without 1 μM NBMPR in transport buffer at a final cell concentration of 0.5 to 1.0 × 10^8 cells/mL. Transport of adenosine (1 μM), thymidine (10 μM), and uridine

Table 1 Fatty acid compositions of the oil formulas

Fatty acids	Oleic acid rich oil (mol% total	Fish oil mol% total
14:0	0.2	6.1
16:0	6.1	10.2
16:1n-7	0.1	10.0
18:0	3.0	1.2
18:1n-9	66.3	8.4
18:1 iso	0.0	2.7
18:2n-6	21.1	3.3
18:3n-3	1.4	1.2
18:4n-3	0.0	4.5
20:1 iso	0.4	1.0
20:4n-6	0.0	1.0
20:4n-3	0.0	1.0
20:5n-3	0.0	32.4
22:5n-3	0.0	2.2
22:6n-3	0.0	12.0
Total saturates	10.5	18.8
Total MUFA	67.0	23.3
Total n-6 PUFA	21.2	4.8
Total n-3 PUFA	1.4	53.2
Ratio n-6/n-3	15.5	0.1

Table 2 Subject characteristics*

Supplement	*n*	Smokers	Weight (kg)	Age (years)	Height (cm)
Fish oil–fed	10	2	83 ± 4	27 ± 1	182 ± 2
Oleic acid–rich oil-fed	10	1	85 ± 3	26 ± 1	181 ± 2

*Subject characteristics did not change over the 6-week study period.

(10 μM) were measured essentially as described by Jarvis et al.[42] except that uptake was terminated by addition of a 2-fold excess volume of cold dilazep (200 μM, a gift from Hoffman La-Roche, Switzerland), and cells were subsequently pelleted through oil. Uptake was initiated by adding 100 μL of cells (with or without NBMPR) to ^{3}H-permeant (all radionucleotides from Dupont, Canada), and uptake was measured over intervals of 0 to 5 sec (3 to 4 tubes/condition) at which point cold dilazep stopper was added, and cells were rapidly pelleted through oil. Permeant solution was aspirated, and the oil layer was washed twice in water. Finally the oil layer was removed and the cell pellet was solubilized in 300 μL of 5% Triton X-100 followed by decolorization with 50 μL of 50% peroxide and liquid scintillation counting in 5 mL of Ecolite (Baxter, Canada). Initial transport rates were estimated by calculating the slopes at 1 sec using computer-generated best fit curves (using the second-order polynomial rate equation and Cricket-graph program).

Fatty acid analysis

Total lipid was extracted from approximately 1 mL of washed red cells (1.0×10^8 cells) in Na^+ buffer by the method of Bligh and Dyer.[43] The phospholipid fraction was separated from other lipids by thin layer chromatography on silica 60 plates (Merck, Germany) in a solvent of heptane/isopropyl ether/acetic acid (60:40:3). The origin containing phospholipids was scraped after visualization with 0.1% aminonaptholsulfonic acid, and fatty acids were methylated after adding 17:0 (3 μg) as an internal standard. Methylated fatty acids were analyzed by gas phase chromatography as described.[44]

Statistics

Data were analyzed by *t*-test for correlated samples using the general linear modeling procedure (SAS/PC V6.04, SAS, Cary, NC). Differences were considered significant at $P < 0.05$.

Results

Transport of nucleosides in erythrocytes

Six weeks of supplementation was chosen since this was the period of time required to achieve maximal incorporation of EPA and DHA into red cell membranes.[34] The transport of three nucleosides, thymidine, uridine, and adenosine, was examined in freshly prepared erythrocytes from subjects on day 0 and again on day 42. Uptake was determined over a time course of 0 to 5 sec, and the initial rates of uptake (from second-order polynomial rate equations) were determined for each subject. Decreases in the NBMPR-sensitive transport rates were observed, regardless of whether subjects were supplemented with oleic acid–rich oil or fish oil (containing predominantly EPA and DHA). Statistical comparison of data from each treatment group demonstrated that there were no differences between fish oil and oleic acid–rich oil-fed subjects at either time point in the study. Therefore, data were pooled for further analysis, and the mean transport rates were determined at day 0 and day 42. Transport rates decreased by 20% for thymidine, 15% for uridine, and 25% for adenosine over the 6-week feeding trial period (*Table 3*).

We also examined the possibility that passive transport across the red cell membrane was modulated by lipid supplementation. *Table 4* shows the rates of passive uptake for adenosine, thymidine, and uridine on day 0 and following 6 weeks of supplementation with the fish and oleic acid–rich oils. Again no differences were found between the treatment groups and data were pooled for analysis. Rates of uptake were low for all nucleosides and not different after oil supplementation. This suggested that the passive movement of nucleosides across the red cell membrane was insensitive to changes in dietary lipid composition.

Fatty acid composition of vegetable and fish oil fed subjects

The fatty acid composition of total phospholipids on day 0 and day 42 for vegetable and fish oil–fed subjects is shown in *Table 5*. There were no changes (either by *t*-test or paired *t*-test, $P < 0.05$) in the fatty acid profile of oleic acid–rich oil-fed subjects when either individual fatty acids were considered or classes of fatty acids were pooled (i.e., n-6 or n-3). Small differences may have been obscured by the substantial variation in the major fatty acids between subjects at day 0 and at day 42 and by the small number of paired scores ($n = 7$). For fish oil supplemented subjects, changes in fatty acid composition as a result of feeding were more dramatic. A significant (25%) drop in arachidonic acid, 20:4n-6, and both its precursor, dihomo-γ-linolenic acid (20:3n-6) and elongation/desaturation product, 22:5n-6, was observed following fish oil feeding. There was a 5.6 mol% decrease in total n-6 fatty acids and a concomitant 4.0 mol% increase n-3 fatty acids. The major fatty acids that contributed to the n-3 increase were 20:5n-3 (eicosapentaenoic acid, EPA) or 22:5n-3 (docosapentaenoic acid, DPA) and 22:6n-3 (docosahexaenoic acid, DHA). The

Table 3 Rates of nucleoside transport in erythrocytes on day 0 and day 42

Nucleoside	Transport rate (pmol/10^7 cells/sec) Day 0	Day 42
Thymidine (10 μM)	11.4 ± 0.3	9.4 ± 0.1*
Uridine (10 μM)	8.30 ± 0.26	7.02 ± 0.09*
Adenosine (1 μM)	1.65 ± 0.04	1.27 ± 0.02*

Initial rates of uptake were determined for each sample on day 0 and then again 42 days after the oil feeding was begun. Averages for all individuals before and after treatment were compared by *t*-test for correlated samples. Differences were considered significant at $P < 0.05$ and are shown by *. Transport rates (pmol/10^7 cells/sec) are shown ± standard error of the mean ($n = 14$).

Table 4 Passive uptake of nucleosides in erythrocytes on day 0 and day 42

	Transport rate (pmol/10^7 cells/sec)	
	Day 0	Day 42
Thymidine (10 μM)	0.033 ± 0.001	0.034 ± 0.004
Uridine (10 μM)	0.025 ± 0.006	0.037 ± 0.006
Adenosine (1 μM)	0.019 ± 0.003	0.021 ± 0.002

Initial rates of uptake were determined in the presence of 1 μM NBMPR for each sample on day 0 and then again 42 days after the oil feeding was begun. Averages for all individuals before and after treatment were compared by *t*-test for correlated samples. Differences were not considered significant ($P > 0.05$). Transport rates (pmol/10^7 cells/sec) are shown ± standard error of the mean ($n = 14$).

net increase in n-3 fatty acids and decrease in n-6 fatty acids resulted in a 2-fold decrease in the n-6/n-3 ratio.

Although changes in fatty acid composition were not statistically significant for oleic acid–fed subjects, when rates of transport of adenosine were correlated with absolute levels of individual fatty acids, in both treatment groups (fish and oleic acid–rich oil), at either day 0 or 42, a significant inverse correlation was found between the absolute level of EPA in phospholipids and the rate of adenosine transport ($r = 0.38$, $n = 28$, $P < 0.05$). The correlation coefficients for thymidine and uridine transport rates and EPA content were -0.21 and -0.24, respectively (NS, $n = 28$, $P > 0.05$).

Discussion

We have shown that supplementation of the diets of human male subjects with oils rich in either oleic acid or omega-3 fatty acids (EPA and DHA) decreases nucleoside transport rates in the erythrocyte. We initially proposed that the changes in nucleoside transport rates were likely due to changes in membrane fatty acid composition. Our in vitro studies in L1210 leukemia cells supported this view since both DHA and EPA supplementation (as purified fatty acids bound to BSA) leads to substantial changes in the fatty acid composition of membrane phospholipids and this correlated with changes in adenosine transport rates[45] and changes in the toxicity of nucleoside drugs.[46] In the feeding trial we report here, substantial changes in total fatty acid composition occurred in fish oil–fed subjects and could be responsible for the changes in nucleoside uptake rates we have observed in these individuals. Activity of the transporter appears to be depressed for all substrates tested: adenosine, thymidine, and uridine. Uridine is not readily phosphorylated by erythrocytes and thus uptake is likely to represent transport specifically. However, adenosine is readily metabolized by erythrocytes so some of the decreased uptake could be due to altered metabolism. Even if this were true, the net result of changes in transport and/or the activity of metabolic enzymes inside the cell would result in a decreased rate of adenosine metabolite production by the red blood cell.

Since fish oil supplementation has been shown to alter red cell membrane fluidity[47–49] one might expect the passive diffusion of nucleosides to be affected by fish and/or oleic acid–rich oil feeding. Because red cells possess only one nucleoside transporter, the facilitated NBMPR-sensitive type, uptake in the presence of 1 μM NBMPR is an index of passive diffusion rates. Our results demonstrate that no change in passive diffusion occurred in either treatment group. This argues that the changes in red cell permeability are selective for protein-mediated transport processes.

Trends toward similar (but less substantive) changes in fatty acid profiles were seen in oleic acid–rich oil-supplemented subjects; however, none of these were significant at $P < 0.05$ (for e.g., day 0 vs. day 42 for EPA, $P = 0.08$). Because the red cell is so long lived (120 days) one possible explanation could have been that the 6-week feeding period was too short to produce substantial changes in lipid composition in oleic acid–rich oil-fed subjects. This seems an unlikely explanation since in a parallel study Turini and coworkers[39] found that the fatty acid composition in platelets, which have a much shorter life span, was also not altered in the oleic acid–rich oil-fed group but was significantly altered in the fish oil–fed group. However, like our results with red cells, platelets from both treatments showed decreased aggregation in response to collagen. Thus, in a different cell population from the same feeding trial, a change in the functional activity of the cell was demonstrated without a change in total phospholipid fatty acid composition or in specific classes of fatty acids. One possibility is that oleic acid–rich oil feeding alters fatty acid composition of a specific phospholipid subclass, another lipid class, or modulates another critical lipid, such as cholesterol content of the erythrocyte membrane. Turini et al.[39] specifically looked at different phospholipid classes for changes in fatty acid composition and did not note differences in platelets isolated on day 0 and day 42 from subjects on the oleic acid–rich supplement. It is, of course, possible that changes in specific phospholipid classes did occur in the erythrocyte following oleic acid–rich oil feeding and that these changes contributed to the changes in nucleoside uptake we observed. Changes in cholesterol content could potentially modulate transporter activity but since platelet plasma cholesterol was unaltered in both treatment groups[39] a change in red cell membrane cholesterol content would not be expected. Another suggestion is that oleic acid may covalently modify membrane or cellular proteins. Both EPA and arachidonic acid have been found covalently attached to platelet proteins and it has been proposed that these modifications regulate protein function/activity.[50] It is conceivable that oleic acid could act similarly although this has yet to be demonstrated.

An interesting finding was that when adenosine uptake rates were plotted as a function of phospholipid EPA content (mol%) a significant inverse correlation was found regardless of time (baseline or 6-weeks post-treatment) or treatment group (fish or oleic acid–rich oil). This suggests that the phospholipid EPA content may be a regulator of nucleoside uptake. In vitro studies utilizing purified EPA at graded concentrations could allow this potential relationship to be better studied.

Table 5 Erythrocyte phospholipid fatty acid composition

	Oleic acid–rich oil (n = 7)		Fish oil (n = 8)	
	Day 0	Day 42	Day 0	Day 42
15:0	0.68 ± 0.07	0.61 ± 0.06	0.68 ± 0.07	0.72 ± 0.18
16:0	21.5 ± 0.4	22.7 ± 1.5	20.5 ± 0.4	21.4 ± 0.4
16:1	0.3 ± 0.2	0.2 ± 0.1	0.5 ± 0.3	1.1 ± 0.6
18:0	5.2 ± 2.1	5.2 ± 2.1	6.8 ± 2.0	7.3 ± 2.3
18:1	15.9 ± 2.5	12.8 ± 0.8	14.2 ± 1.3	14.1 ± 0.9
18:2n-6	11.6 ± 1.3	11.8 ± 1.3	11.3 ± 1.2	10.9 ± 1.6
18:3n-6	2.5 ± 1.5	4.8 ± 1.3	3.8 ± 1.7	3.2 ± 1.0
18:3n-3	0.4 ± 0.1	0.26 ± 0.06	0.23 ± 0.06	0.17 ± 0.04
18:4n-3	0.08 ± 0.04	0.10 ± 0.04	0.12 ± 0.05	0.13 ± 0.07
20:0	0.45 ± 0.04	0.52 ± 0.04	0.45 ± 0.05	0.49 ± 0.06
20:1	0.4 ± 0.1	0.40 ± 0.05	0.33 ± 0.07	0.36 ± 0.04
20:2n-6	0.40 ± 0.08	0.25 ± 0.03†	0.41 ± 0.16	0.46 ± 0.26
20:3n-6	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	0.75 ± 0.10*
20:4n-6	9.5 ± 1.8	9.0 ± 0.9	11.0 ± 0.9	8.3 ± 1.1*
20:3n-3	0.2 ± 0.1	0.26 ± 0.07	0.22 ± 0.07	0.26 ± 0.08
20:4n-3	1.0 ± 1.0	0.2 ± 0.2	0.19 ± 0.16	0.11 ± 0.06
20:5n-3	0.5 ± 0.2	1.4 ± 0.5‡	0.43 ± 0.08	2.4 ± 0.5*
22:0	1.8 ± 0.3	1.9 ± 0.3	1.8 ± 0.4	2.0 ± 0.1
22:1	0.21 ± 0.04	0.22 ± 0.05	0.40 ± 0.06	0.26 ± 0.06
22:2n-6	0.07 ± 0.04	0.23 ± 0.09§	0.25 ± 0.18	0.10 ± 0.05
22:4n-6	3.0 ± 0.3	2.5 ± 0.4	2.8 ± 0.6	3.1 ± 1.2
22:5n-6	0.5 ± 0.1	0.6 ± 0.1	0.68 ± 0.09	0.44 ± 0.10*
22:5n-3	1.2 ± 0.2	1.7 ± 0.3	2.0 ± 0.2	3.1 ± 0.4*
22:6n-3	2.4 ± 0.4	3.0 ± 0.5	3.2 ± 1.3	4.4 ± 0.4*
24:0	5.1 ± 0.4	5.9 ± 0.7	4.9 ± 1.3	5.0 ± 1.0
24:1	5.7 ± 0.6	5.6 ± 0.6	5.3 ± 1.2	5.6 ± 1.2
saturates	34.8 ± 3.0	36.8 ± 2.8	35.1 ± 2.5	36.9 ± 1.9
MUFAs	22.5 ± 2.6	19.2 ± 1.0	20.7 ± 0.5	21.4 ± 0.7
n-6 PUFAs	28.6 ± 2.8	30.3 ± 2.7	31.4 ± 2.4	27.2 ± 3.2*
n-3 PUFAs	5.8 ± 1.0	6.9 ± 1.0	6.4 ± 0.6	10.6 ± 1.0*
n-6/n-3 ratio	4.9 ± 0.6	4.4 ± 0.8	4.9 ± 0.8	2.6 ± 0.7*

Values given are for mol% of total fatty acids in the purified phospholipids.
*Values on day 42 were statistically different from baseline values on day 1 by paired t-test, $P < 0.05$.
Symbols in column two indicate differences from day 0: †P = 0.10; ‡P = 0.08; §P = 0.13. Unidentified fatty acids accounted for 8.3, 6.8, 6.4, and 3.9 mol%, for columns 2 to 5, respectively.

In conclusion, our results demonstrate that both fish oil and oleic acid–rich vegetable oil feeding have the potential to modify erythrocyte membrane transporter activity. The precise mechanisms by which the individual omega-3 and monounsaturated fatty acids mediate these changes remain to be elucidated. The decreased rates of nucleoside uptake reported in this study could have important implications. Since the erythrocyte likely represents the major reservoir and metabolic compartment, for adenosine released into the blood by the liver, other blood cells and vascular endothelial cells, changes in transporter activity could have significant effects on vascular tone and platelet reactivity.[51] Following adenosine release, a decrease in the uptake of adenosine by the erythrocyte would allow adenosine concentrations to remain higher for a longer period of time and thereby promote vasodilation and inhibit platelet aggregation. Decreased rates of uptake would also delay the release of adenosine metabolites by the erythrocytes. Thus, we can add the modulation of adenosine metabolism to the multiple physiologic responses already described for oleic acid and fish oil–rich diets. The clinical value of such a finding remains to be determined because the effects of fatty acids on other critical cells and tissues needs to be assessed. The possibility that supplements such as those described in this study modulate nucleoside transport, adenosine receptor function, or 5′-nucleotidase activity, all of which contribute to the adenosine response, needs to be examined in other cell types or tissues involved in the cardiovascular response.

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