

ORIGINAL ARTICLE

# Arachidonate 5-Lipoxygenase Promoter Genotype, Dietary Arachidonic Acid, and Atherosclerosis

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## ABSTRACT

### BACKGROUND

Leukotrienes are inflammatory mediators generated from arachidonic acid (polyunsaturated n-6 fatty acid) by the enzyme 5-lipoxygenase. Since atherosclerosis involves arterial inflammation, we hypothesized that a polymorphism in the 5-lipoxygenase gene promoter could relate to atherosclerosis in humans and that this effect could interact with the dietary intake of competing 5-lipoxygenase substrates.

### METHODS

We determined 5-lipoxygenase genotypes, carotid-artery intima-media thickness, and markers of inflammation in a randomly sampled cohort of 470 healthy, middle-aged women and men from the Los Angeles Atherosclerosis Study. Dietary arachidonic acid and marine n-3 fatty acids (including a competing 5-lipoxygenase substrate that reduces the production of inflammatory leukotrienes) were measured with the use of six 24-hour recalls of food intake.

### RESULTS

Variant 5-lipoxygenase genotypes (lacking the common allele) were found in 6.0 percent of the cohort. Mean ( $\pm$ SE) intima-media thickness adjusted for age, sex, height, and racial or ethnic group was increased by  $80 \pm 19 \mu\text{m}$  (95 percent confidence interval, 43 to 116;  $P < 0.001$ ) among carriers of two variant alleles, as compared with carriers of the common (wild-type) allele. In multivariate analysis, the increase in intima-media thickness among carriers of two variant alleles ( $62 \mu\text{m}$ ,  $P < 0.001$ ) was similar in this cohort to that associated with diabetes ( $64 \mu\text{m}$ ,  $P = 0.01$ ), the strongest common cardiovascular risk factor. Increased dietary arachidonic acid significantly enhanced the apparent atherogenic effect of genotype, whereas increased dietary intake of n-3 fatty acids blunted the effect. Finally, the plasma level of C-reactive protein, a marker of inflammation, was increased by a factor of 2 among carriers of two variant alleles as compared with that among carriers of the common allele.

### CONCLUSIONS

Variant 5-lipoxygenase genotypes identify a subpopulation with increased atherosclerosis. The observed diet-gene interactions further suggest that dietary n-6 polyunsaturated fatty acids promote, whereas marine n-3 fatty acids inhibit, leukotriene-mediated inflammation that leads to atherosclerosis in this subpopulation.

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**E**ICOSANOIDS ARE LIPID MEDIATORS OF inflammation and hypersensitivity reactions,<sup>1</sup> and arachidonate 5-lipoxygenase is the key enzyme in the oxidative biosynthesis of a class of paracrine and autocrine eicosanoids known as leukotrienes.<sup>2</sup> The dihydroxy leukotriene B<sub>4</sub> is a potent leukocyte chemoattractant, whereas the cysteinyl leukotrienes increase vascular permeability and promote contraction of vascular smooth muscle.<sup>3</sup> The cysteinyl leukotrienes have been linked to asthma,<sup>4</sup> and 5-lipoxygenase promoter genotypes have been identified that interacted strongly with the effects of 5-lipoxygenase inhibition in patients with asthma.<sup>5,6</sup> This drug-gene interaction suggested a functional difference in the 5-lipoxygenase pathway between carriers of at least one common (wild-type) allele and carriers of two variant alleles.

Atherosclerosis is a chronic inflammatory process involving the recruitment and accumulation of monocytes, macrophages, and dendritic cells in artery walls, where they become loaded with modified and aggregated low-density lipoproteins (LDLs).<sup>7,8</sup> Molecular determinants of the pathologic chronicity of this process are unknown.

The 5-lipoxygenase pathway has been linked to atherosclerosis in mouse models<sup>9,10</sup> and in a histologic study in humans.<sup>11</sup> These findings suggested the hypothesis that variation in the 5-lipoxygenase promoter could alter eicosanoid-mediated inflammatory circuits in the artery wall and promote atherogenesis. Since the intake of arachidonic acid increases<sup>12,13</sup> and the intake of marine n-3 fatty acids reduces<sup>14</sup> the production of leukotriene B<sub>4</sub> in human monocytes, we further hypothesized that dietary intake of competing 5-lipoxygenase substrates would interact with an atherogenic effect of genotype. We investigated these hypotheses by relating carotid-artery intima-media thickness to 5-lipoxygenase promoter genotypes and dietary intake in the Los Angeles Atherosclerosis Study.<sup>15</sup> Intima-media thickness is an indicator of systemic atherosclerosis that strongly predicts atherothrombotic events.<sup>16</sup>

## METHODS

### COHORT

The cohort of 573 women (age, 45 to 60 years) and men (age, 40 to 60 years) was free of diagnosed cardiovascular disease when randomly sampled from an employee population.<sup>17</sup> Hispanic subjects and

smokers were oversampled, and the participation rate was 85 percent. Base-line examinations in 1995–1996 were followed by two examinations at 1.5-year intervals, in which buffy coat was collected (from 500 subjects) for DNA extraction.

The study protocol was approved by the institutional review board of the Keck School of Medicine in Los Angeles. All subjects provided written informed consent.

### CAROTID INTIMA-MEDIA THICKNESS

The degree of atherosclerosis in the posterior wall of the common carotid arteries was estimated bilaterally in the base-line examination as intima-media thickness by high-resolution B-mode ultrasonography, as described previously.<sup>18</sup> The coefficient of variation was 2.8 percent for repeated scans by different sonographers.<sup>18</sup>

### GENOTYPING

DNA was isolated from 500 subjects, and the number of tandem Sp1 binding motifs (5'GGGCGG3') in the 5-lipoxygenase (ALOX5) promoter was determined in 470 subjects according to previously described methods.<sup>5</sup> This group included non-Hispanic whites (55.1 percent), Hispanic subjects (29.6 percent), Asian or Pacific Islanders (7.7 percent), blacks (5.3 percent), and other groups (2.3 percent). The genotype of 30 specimens could not be determined owing to polymerase-chain-reaction failure. The resulting six alleles had relative frequencies of 2.9 percent, 13.1 percent, 80.5 percent, 2.8 percent, 0.5 percent, and 0.2 percent for three, four, five, six, seven, or eight tandem Sp1 motifs, respectively. Variant alleles involved deletions (one or two) or additions (one, two, or three) of Sp1 motifs to the five tandem motifs in the common allele. The distribution of the genotypes did not significantly deviate from that expected by random combination of variant and common alleles within any of the racial or ethnic groups ( $P \geq 0.05$  according to Hardy-Weinberg equilibrium  $\chi^2$ ).

### LABORATORY MEASUREMENTS

Data on serum lipid levels were available for all 470 genotyped subjects. Plasma levels of C-reactive protein, interleukin-6, interleukin-8, and tumor necrosis factor  $\alpha$  were measured at base line in 27 of the 28 carriers of two variant alleles and in 38 matched carriers of the common allele, with the use of commercially available, high-sensitivity kits. Fatty acids

in frozen plasma were also measured in this subgroup (at the Heber Laboratory at UCLA) for validation of dietary measurements.

#### DIETARY INTAKE

Six 24-hour recalls of food intake were obtained from the subjects over a period of 1.5 years with the use of the Nutrition Data System.<sup>19</sup> Fatty-acid intake (expressed as grams per 1000 kcal) was averaged over the six dietary recalls. Intakes of long-chain n-3 polyunsaturated fatty acids (eicosapentaenoic and docosahexaenoic acids) were summed, owing to the high correlation between the intake of these two fatty acids (rank-order correlation, 0.85). The intakes of arachidonic acid and its metabolic precursor (linoleic acid) were only weakly correlated ( $r=0.15$ ) and were estimated separately. Plasma and tissue levels of linoleic acid and long-chain n-3 polyunsaturated fatty acids correlate with dietary intake of corresponding fatty acids,<sup>20</sup> and these associations were estimated in a validation subsample of 66 subjects. Spearman correlations between dietary intake (expressed as grams per 1000 kcal) and the corresponding fatty acid in plasma (expressed as a percentage of total fatty acids) were 0.53 ( $P<0.001$ ) for eicosapentaenoic plus docosahexaenoic acids and 0.32 ( $P=0.01$ ) for linoleic acid.

#### STATISTICAL ANALYSIS

Adjusted means and P values for differences between genotype groups were estimated at the mean value of covariates by least-squares regression. The relative odds of an elevated intima-media thickness were estimated by ordinal logistic regression with the use of deciles of intima-media thickness as the ordinal outcome. For figures depicting relations according to three ordinal ranked groups, reported P values are from models with continuous ordinal variables. The covariates in statistical models relating intima-media thickness to genotype were age, interaction of genotype with age (centered at 50 years of age), sex, height, and racial or ethnic group (model 1); all factors in model 1 plus cigarette-smoking status (currently, formerly, or never), level of physical activity, dietary intake of saturated fat (expressed as a percentage of energy intake), and intake of alcohol (model 2, behavioral risk factors); and all factors in model 2 plus serum cholesterol level, serum high-density lipoprotein (HDL) cholesterol level, systolic blood pressure, body-mass index (the weight in kilograms divided by the square of

the height in meters), presence or absence of diabetes (type 1 or 2), use of antihypertensive medication, and use of lipid-lowering medication (model 3, biologic risk factors and preventive treatments).

The primary analyses compared carriers of at least one common allele with carriers of two variant alleles. This categorization was derived from a pharmacogenetic interaction involving these two genotype groups.<sup>6</sup> Some a posteriori comparisons for additional 5-lipoxygenase genotype subgroups are also presented for the purpose of hypothesis generation.

## RESULTS

Genotyping yielded 442 carriers of the 5-lipoxygenase common allele (94.0 percent) and 28 carriers of two variant alleles (6.0 percent). Major cardiovascular risk factors are presented for the cohort in Table 1, according to promoter genotype. No significant differences between carriers of the common allele and carriers of two variant alleles were apparent. However, the prevalence of variant genotypes did differ across racial and ethnic groups ( $P<0.001$  by the chi-square test), with higher prevalences among Asians or Pacific Islanders (19.4 percent), blacks (24.0 percent), and other racial or ethnic groups (18.2 percent) than among Hispanic subjects (3.6 percent) and non-Hispanic whites (3.1 percent).

**Table 1. Major Cardiovascular Risk Factors According to 5-Lipoxygenase Genotype.\***

Variable	Carriers of the Common Allele (N=442)	Carriers of Two Variant Alleles (N=28)
Age (yr)	50.0±4.6	49.3±4.8
Systolic blood pressure (mm Hg)	128±16	132±14
Serum cholesterol (mmol/liter)		
Total	5.6±0.7	5.4±1.0
HDL	1.5±0.3	1.4±0.4
Female sex (%)	46.8	42.9
Current smoking (%)	24.2	21.4
Former smoking (%)	26.5	25.0
Diabetes (type 1 or 2) (%)	2.3	3.6

\* Plus-minus values are means ±SD. There were no significant differences between groups with the use of a t-test for continuous variables and the chi-square test for categorical variables. The common (wild-type) 5-lipoxygenase allele has five tandem Sp1 motifs. To convert values for cholesterol to milligrams per deciliter, divide by 0.02586.

**5-LIPOXYGENASE POLYMORPHISM AND ATHEROSCLEROSIS**

Means and medians of carotid intima-media thickness according to 5-lipoxygenase genotype are presented in Table 2. The significance level of the unadjusted elevation of intima-media thickness in the group with two variant alleles was confirmed by nonparametric bootstrap analysis. After adjustment for age, sex, height, and racial or ethnic group, the mean ( $\pm$ SE) intima-media thickness was elevated by  $80\pm 19\ \mu\text{m}$  among carriers of two variant alleles, as compared with carriers of the common allele (95 percent confidence interval, 43 to 116;  $P<0.001$ ) (Table 2). This elevation remained significant after adjustment for behavioral risk factors ( $78\pm 19\ \mu\text{m}$ ,  $P<0.001$ ) and biologic confounders or mediators and preventive treatments ( $62\pm 17\ \mu\text{m}$ ,  $P<0.001$ ) (Table 2). The magnitude of the apparent genotype effect in this last model is similar to that associated with diabetes in this cohort ( $64\pm 26\ \mu\text{m}$ ,  $P=0.01$ ) and larger than that associated with current smoking ( $45\pm 11\ \mu\text{m}$ ,  $P<0.001$ ).

This apparent atherogenic effect did not significantly interact with sex or smoking status, but it did increase with age ( $P$  for interaction= $0.04$ ). The intima-media thickness was greater in the group with two variant alleles within all five racial and ethnic categories. These differences between genotypes were greatest among blacks and smallest among non-Hispanic whites, with the differences in other groups being intermediate (data not shown). However, these differences were not statistically significant ( $P=0.26$ ).

The relative magnitude of this 5-lipoxygenase genotype association was estimated with the use of ordinal logistic regression. After adjustment for age, sex, height, and racial or ethnic group, the odds of increased wall thickness were elevated by a factor of 4 among carriers of two variant alleles as compared with carriers of the common allele (odds ratio, 4.1; 95 percent confidence interval, 2.1 to 8.2;  $P<0.001$ ). Adjustment for numerous potential confounders did not markedly attenuate this relation (odds ratio, 3.7;  $P<0.001$ ).

The association between genotype and intima-media thickness was further investigated in five 5-lipoxygenase genotype groups derived from combinations of common (W), deletion (D), and addition (A) alleles: DD (18 subjects), DA (9), WD (105), WA (22), and WW (315); the AA genotype was observed in only 1 subject (intima-media thickness,  $661\ \mu\text{m}$ ). The differences among the five genotype groups confirmed the presence of a recessive pattern of effects (Fig. 1).

**DIET-GENE INTERACTIONS**

If the observed increase in the intima-media thickness in the 5-lipoxygenase variants was due to increased production of leukotrienes (e.g., leukotriene  $B_4$ ), then increased availability of the 5-lipoxygenase substrate arachidonic acid and its metabolic precursor (linoleic acid) could amplify the atherogenic effect of the variant genotypes. Similarly, increased intake of eicosapentaenoic and docosahexaenoic acids could reduce the production of inflammatory leukotrienes and inhibit this effect.<sup>14</sup>

Increased intima-media thickness was significantly associated with intake of both arachidonic acid ( $P$  for trend  $<0.001$ ) and linoleic acid ( $P$  for trend= $0.03$ ) among carriers of two variant alleles (Fig. 2A and 2B) but not among carriers of the common allele ( $P$  values for interaction are listed in Fig. 2). In contrast, the intake of marine n-3 fatty acids was significantly and inversely associated with intima-media thickness only among carriers of two

**Table 2. Carotid Intima-Media Thickness According to 5-Lipoxygenase Genotype.\***

Variable	Intima-Media Thickness		P Value†
	Carriers of the Common Allele (N=442)	Carriers of Two Variant Alleles (N=28)	
	$\mu\text{m}$		
No covariates			
Mean $\pm$ SD	661 $\pm$ 95	736 $\pm$ 141	<0.001
Bootstrap P value			<0.001
Median	641	725	0.004
Minimum	428	526	
Maximum	1096	1076	
Multivariate analysis			
Model 1 (mean $\pm$ SE)	661 $\pm$ 4	740 $\pm$ 18	<0.001
Model 2 (mean $\pm$ SE)	661 $\pm$ 4	739 $\pm$ 18	<0.001
Model 3 (mean $\pm$ SE)	662 $\pm$ 4	724 $\pm$ 16	<0.001

\* Model 1 included the following covariates: age, sex, height, and racial or ethnic group (non-Hispanic white, Hispanic, Asian or Pacific Islander, black, and other). Model 2 included all the covariates in model 1 as well as the following behavioral risk factors: smoking status, level of physical activity, dietary intake of saturated fat, and intake of alcohol. Model 3 included all the covariates listed in model 2 as well as the following biologic risk factors and preventive pharmacologic treatments: serum cholesterol, serum HDL cholesterol, systolic blood pressure, body-mass index, presence or absence of diabetes, use of antihypertensive medication, and use of lipid-lowering medication.

† P values for differences between means were computed by regression analysis. The P value for the difference in medians was calculated with the use of the Kruskal-Wallis test. The bootstrap P value was estimated with 1 million samplings.

variant alleles ( $P$  for trend=0.007) (Fig. 2C). Diet-gene interactions were specific to these fatty acids and were not observed for dietary intake of mono-unsaturated fat (Fig. 2D), saturated fat (Fig. 2E), or other measured fatty acids (data not shown).

#### LDL-GENE INTERACTION

LDL cholesterol levels in plasma did not differ significantly between 5-lipoxygenase carriers of two variant alleles and carriers of the common allele ( $P=0.33$ ), nor were there significant differences across the five genotype groups ( $P=0.21$  by analysis of variance) (Fig. 3A). However, the LDL cholesterol level was a more potent atherogenic factor among carriers of two variant alleles than among carriers of the common allele (Fig. 3B).

#### 5-LIPOXYGENASE POLYMORPHISM AND INFLAMMATION

The level of C-reactive protein was increased by a factor of 2 among carriers of two variant alleles, as compared with carriers of the common allele (mean, 2.6 vs. 1.3 mg per liter;  $P=0.007$ ), and levels of interleukin-6 and tumor necrosis factor  $\alpha$  were marginally increased by 32 percent ( $P=0.07$ ) and 17 percent ( $P=0.11$ ), respectively. The level of interleukin-8 was decreased by 3 percent among carriers of two variant alleles ( $P=0.77$ ). These analyses of logarithmically transformed values included the covariates age, sex, height, smoking status, and racial or ethnic group.

#### DISCUSSION

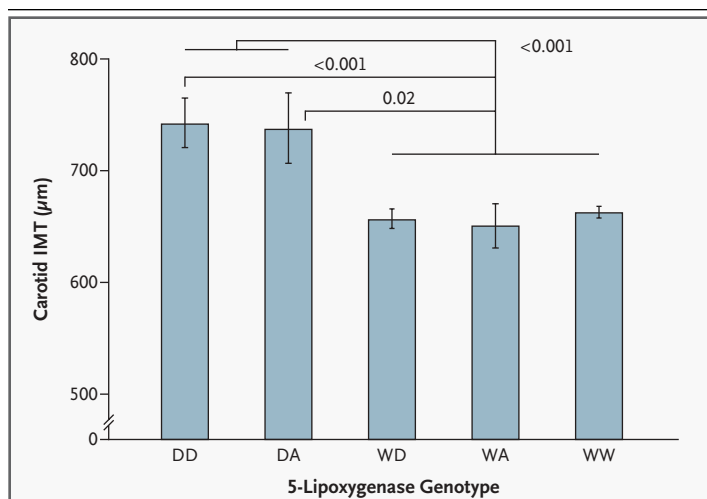
We found a large increase in carotid intima-media thickness among carriers of two variant 5-lipoxygenase promoter alleles as compared with carriers of the common allele. After multivariate adjustment, the apparent atherogenic effect remained as large as that associated with diabetes. This association was also robust across racial and ethnic groups that differed in the prevalence of variant genotypes. This strong association contrasts with weak associations between polymorphic variation in other inflammatory-pathway genes and cardiovascular disease outcomes.<sup>21-24</sup>

The diet-gene interactions we observed suggest an effect of genotype on atherosclerosis mediated by the 5-lipoxygenase pathway. Increased dietary intake of n-6 fatty acids (arachidonic acid and its metabolic precursor, linoleic acid) was associated with increased severity of atherosclerosis only among carriers of two variant alleles. Such an interaction

would be expected if, for example, the production of leukotrienes in the artery wall was increased and triggered atherosclerosis in the variant group. Arachidonic acid is the primary substrate for 5-lipoxygenase, and increased intakes of linoleic acid and arachidonic acid enhance the production of leukotrienes.<sup>12,13</sup> This increase could induce an atherogenic chronicity of inflammatory circuits in the artery wall.<sup>11,25</sup>

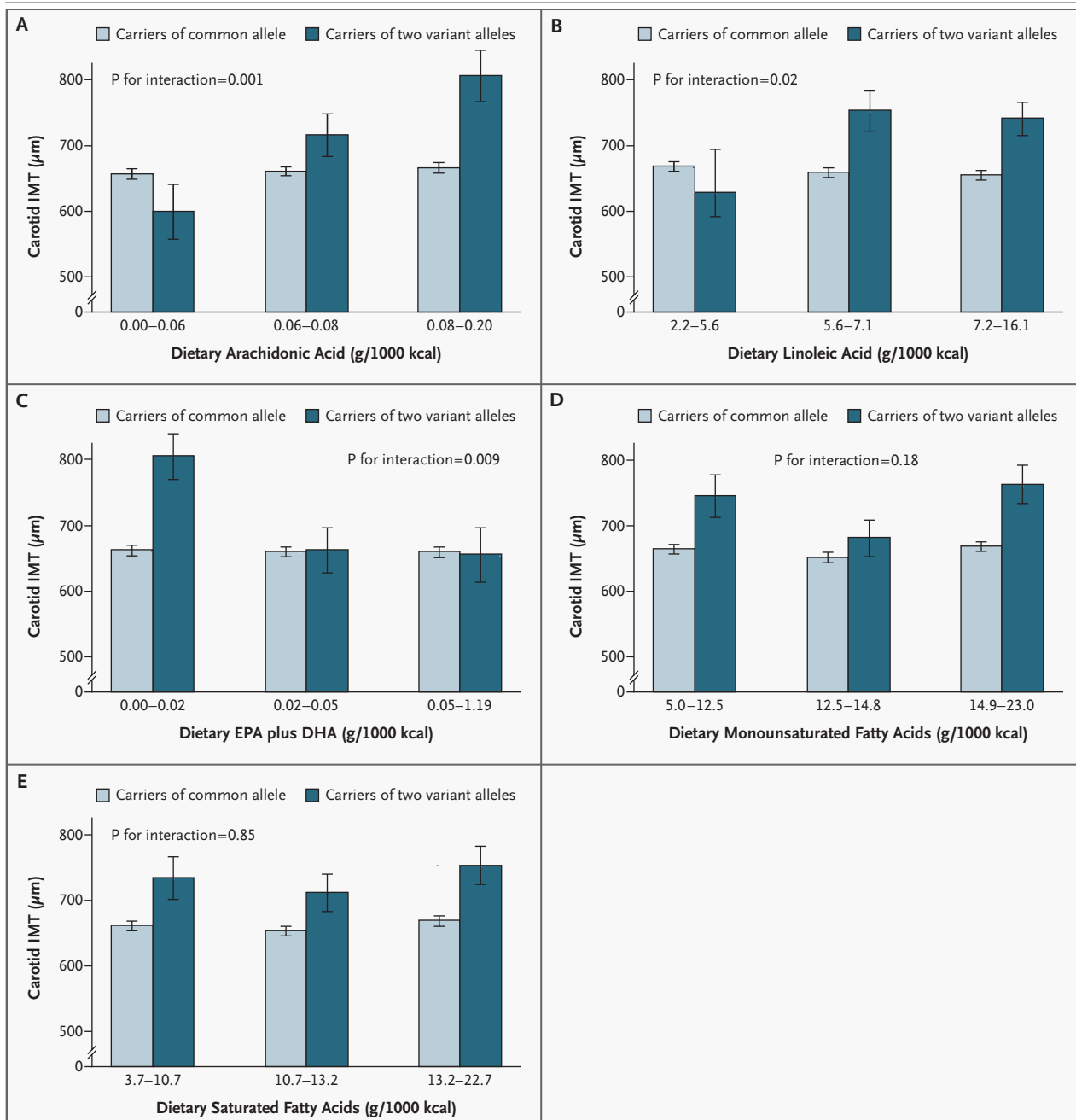
We also found that increased dietary intake of marine n-3 fatty acids blunted the apparent atherogenic effect of the variant genotypes. This interaction was also suggestive of a leukotriene-mediated effect, since eicosapentaenoic acid is a competing substrate for 5-lipoxygenase. Feeding eicosapentaenoic acid and docosapentaenoic acid to humans reduces the production of leukotriene B<sub>4</sub> by activated monocytes.<sup>14,26,27</sup> The intake of marine n-3 fatty acids shifts the production of leukotrienes from the more active B<sub>4</sub> form to the less active B<sub>5</sub> form<sup>28,29</sup> and may also induce the production of other anti-inflammatory mediators.<sup>30</sup> Involvement of the 5-lipoxygenase pathway in these diet-gene interactions was further implicated by the lack of such interactions with other dietary fatty acids.

There is considerable evidence that fish-oil intake protects against sudden death from cardiac causes<sup>31,32</sup> — an antiarrhythmic effect that could



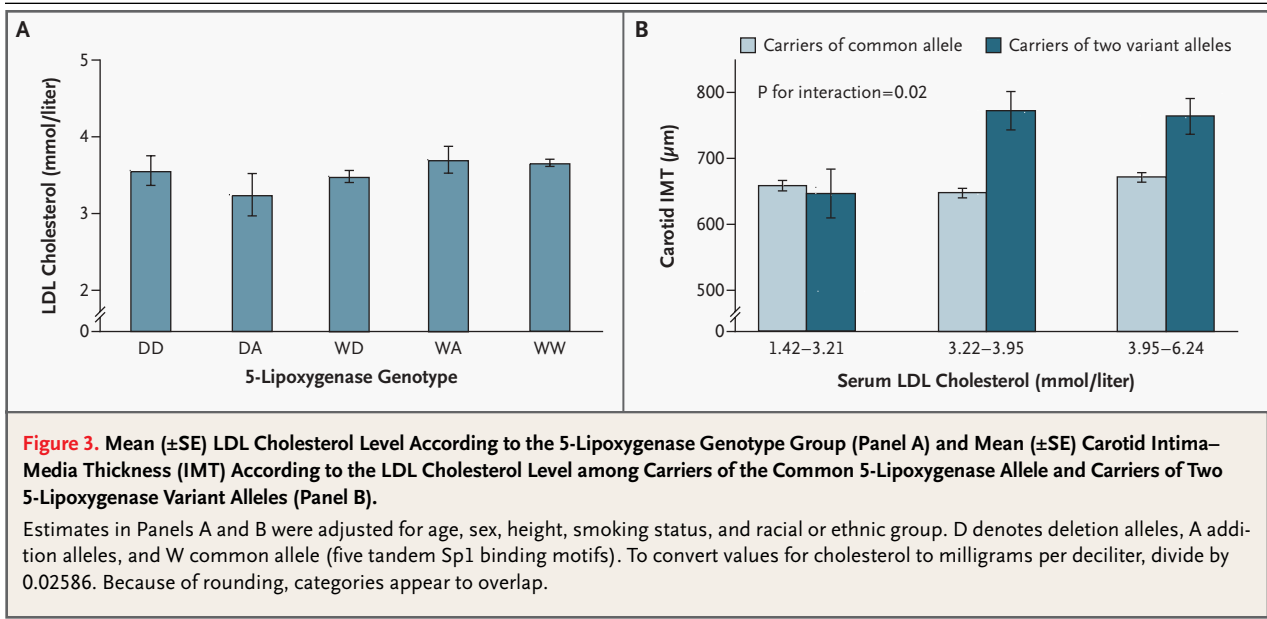
**Figure 1. Mean ( $\pm$ SE) Carotid Intima-Media Thickness (IMT) in Five 5-Lipoxygenase Genotype Groups.**

Means were adjusted for age, sex, height, racial or ethnic group, smoking status, level of physical activity, dietary intake of saturated fat, and intake of alcohol by analysis of covariance. D denotes deletion alleles, A addition alleles, and W common allele (five tandem Sp1 binding motifs). P values are for the differences between indicated genotype groups.



**Figure 2.** Diet–Gene Interactions between Dietary Intake of Arachidonic Acid (Panel A), Linoleic Acid (Panel B), Eicosapentaenoic Acid (EPA) plus Docosahexaenoic Acid (DHA) (Panel C), Monounsaturated Fatty Acids (Panel D), or Saturated Fatty Acids (Panel E) and the Effect of 5-Lipoxygenase Promoter Genotype on Carotid Intima–Media Thickness (IMT).

All interactions were adjusted for age, sex, height, smoking status, and racial or ethnic group; the interactions shown in Panels A, B, and C were adjusted for one another. Arachidonic acid is 5,8,11,14-eicosatetraenoic acid (20:4n–6); linoleic acid is 9,12-octadecadienoic acid (18:2n–6); eicosapentaenoic acid is 5,8,11,14,17-eicosapentaenoic acid (20:5n–3); and docosahexaenoic acid is 4,7,10,13,16,19-docosahexaenoic acid (22:6n–3). Means (±SE) are shown. Because of rounding, categories appear to overlap.



be mediated by cysteinyl leukotrienes.<sup>33</sup> Taken together, our findings suggest an antiatherosclerotic effect of fish oils among carriers of two 5-lipoxygenase variant alleles and are consistent with the occurrence of increased leukotriene production in this group. The finding that LDL cholesterol was more atherogenic among carriers of two variant alleles is consistent with enhanced LDL-mediated atherosclerosis in that group,<sup>34</sup> and among these subjects the C-reactive protein levels, which increased by a factor of two, were consistent with the presence of markedly greater chronic arterial inflammation in this group.<sup>35</sup>

Although data on the 5-lipoxygenase pathway and atherosclerosis are limited, available evidence from two studies in animals and a histologic study in humans is consistent with the hypothesis that increased leukotriene production has an atherogenic effect. The extent of atherosclerosis in the aortic arch was greatly reduced in susceptible mice carrying one null 5-lipoxygenase allele, as compared with the extent among carriers of two functional alleles,<sup>9</sup> suggesting that this inflammatory pathway is important in atherosclerosis. In a second study, foam-cell formation was reduced in three strains of atherosclerosis-susceptible mice treated with a leukotriene-B<sub>4</sub>-receptor antagonist.<sup>10</sup> Third, a recent histologic study in humans found an abundance of 5-lipoxygenase (but not 15-lipoxygenase) in macrophages and foam cells, dendritic cells, and artery-wall cells from atherosclerotic lesions.<sup>11</sup>

Combining these findings with those of recent studies of leukotriene receptors expressed by endothelial cells and macrophages,<sup>25,36</sup> Habenicht's group has proposed a model of leukotriene-mediated vascular inflammation in atherosclerosis.<sup>11,25</sup> In this model, leukotrienes produced by macrophages and dendritic cells in the artery wall have autocrine effects and paracrine effects on endothelial cells, lymphocytes, smooth-muscle cells, and other macrophages or dendritic cells. Up-regulation of this "inflammatory circuit" by environmental or genetic factors would promote atherosclerosis by enhancing the known effects of leukotrienes on the recruitment of leukocytes, endothelial-cell dysfunction, intimal edema, the proliferation of smooth-muscle cells, and immune reactivity.<sup>11</sup> This model suggests a mechanism whereby increased expression of the 5-lipoxygenase gene or activity of the enzyme in carriers of variant genotypes leads to increased carotid intima-media thickness.

Although a previously reported drug-gene interaction suggested that variant 5-lipoxygenase genotypes have a strong effect on function,<sup>6</sup> and the gene-diet interactions found in our study are consistent with a hypothesis of increased leukotriene production among promoter variants, findings in *in vitro* studies of gene expression do not provide support for such a hypothesis.<sup>5,37</sup> Experiments with *drosophila* SL2 (Schneider) cells found increased reporter construct activity for an addition allele (as compared with the common allele), but activity was

reduced with transfection of deletion alleles.<sup>37</sup> Reporter constructs in HeLa cells showed a different pattern: gene expression was reduced for both addition and deletion alleles.<sup>5</sup> However, these promoter-reporter construct studies in nonhuman cells (Schneider) and in tumor cells that do not express 5-lipoxygenase (HeLa) may not reflect the *in vivo* process of vascular inflammation. Only certain types of cells express the 5-lipoxygenase gene, apparently as a result of different patterns of methylation,<sup>38</sup> and expression of the gene is regulated by activating factors. Moreover, Serio and colleagues found that the expression of the leukotriene C<sub>4</sub> synthase gene (a leukotriene-pathway gene) in HeLa cells was not regulated in the same manner as that observed in a monocyte-like cell line.<sup>39</sup> Another investigation of the effect of this polymorphism on leukotriene production by human eosinophils found that variant promoter genotypes only showed increased leukotriene production when cells were stimulated by a calcium ionophore and cyclooxygenase pathways were inhibited.<sup>40</sup> These findings suggest that the effect of variation in the 5-lipoxygenase promoter sequence of tandem Sp1 motifs on leukotriene production in the artery wall may only be detectable in human macrophages and dendritic cells under conditions that mimic the intimal microenvironment.

If the 5-lipoxygenase pathway in cells involved in atherosclerosis is down-regulated among carriers of two variant alleles, as suggested by the reduction in reporter construct activity in HeLa cells, then our observation of increased atherosclerosis among such subjects appears paradoxical. However, inflammatory pathways are redundant and interacting,<sup>41</sup> and down-regulation of the 5-lipoxygenase pathway can result in up-regulation of other eicosanoid pathways.<sup>42</sup> Other candidate eicosanoid pathways for the promotion of atherosclerosis include the 15-lipoxygenase and cyclooxygenase 2 pathways,<sup>43,44</sup> which are also differentially affected by dietary intake of n-6 and marine n-3 polyunsaturated fatty acids.<sup>29,45</sup> However, evidence of the atherogenic effects of these other eicosanoid pathways is

mixed,<sup>11,46,47</sup> and n-3 polyunsaturated fatty acids have a much stronger effect on leukotriene production than on other eicosanoids.<sup>45</sup>

Another possible mechanism linking down-regulation of 5-lipoxygenase to atherosclerosis in carriers of variant genotypes would involve reduced transcellular biosynthesis of antiinflammatory 5-lipoxygenase products such as lipoxins.<sup>48</sup> However, such a model is inconsistent with the diet-gene interactions we observed and with the substantial reduction of atherosclerosis in 5-lipoxygenase-deficient mice.<sup>9</sup>

Weaknesses of our study stem from its observational design. The 5-lipoxygenase polymorphic variation could be confounded by unmeasured environmental or genetic factors. The large magnitude of the apparent effect of the genotype relative to other risk factors for atherosclerosis makes confounding with known risk factors unlikely. Although the possibility of linkage disequilibrium with other polymorphisms clearly cannot be ruled out, the confounding polymorphism must have a profound effect on atherosclerosis. Furthermore, the observed diet-gene interactions provide a clear link between the apparent atherogenic polymorphic effect and pathways affected by the intake of n-3 and n-6 polyunsaturated fatty acids.

If replicated, our findings would constitute clear evidence that genetic variation in an inflammatory pathway,<sup>7</sup> and the leukotriene pathway in particular,<sup>49</sup> can trigger atherogenesis in humans. These findings could lead to new dietary and targeted molecular approaches to the prevention and treatment of cardiovascular disease according to genotype, particularly in populations of non-European descent.

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#### REFERENCES

1. Funk CD. Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science* 2001;294:1871-5.
2. Samuelsson B. Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. *Science* 1983;220:568-75.
3. Samuelsson B, Dahlen SE, Lindgren JA, Rouzer CA, Serhan CN. Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. *Science* 1987;237:1171-6.
4. Drazen JM, Israel E, O'Byrne PM. Treatment of asthma with drugs modifying the leukotriene pathway. *N Engl J Med* 1999;340:197-206. [Errata, *N Engl J Med* 1999;340:663, 341:1632.]
5. In KH, Asano K, Beier D, et al. Naturally occurring mutations in the human 5-lipoxygenase gene promoter that modify transcription factor binding and reporter gene transcription. *J Clin Invest* 1997;99:1130-7.
6. Drazen JM, Yandava CN, Dube L, et al. Pharmacogenetic association between ALOX5 promoter genotype and the response to anti-asthma treatment. *Nat Genet* 1999;22:168-70.
7. Ross R. Atherosclerosis — an inflammatory disease. *N Engl J Med* 1999;340:115-26.
8. Lusis AJ. Atherosclerosis. *Nature* 2000;407:233-41.

9. Mehrabian M, Allayee H, Wong J, et al. Identification of 5-lipoxygenase as a major gene contributing to atherosclerosis susceptibility in mice. *Circ Res* 2002;91:120-6. [Erratum, *Circ Res* 2002;91(8):e27.]
10. Aiello RJ, Bourassa PA, Lindsey S, Weng W, Freeman A, Showell HJ. Leukotriene B4 receptor antagonism reduces monocytic foam cells in mice. *Arterioscler Thromb Vasc Biol* 2002;22:443-9.
11. Spanbroek R, Grabner R, Lotzer K, et al. Expanding expression of the 5-lipoxygenase pathway within the arterial wall during human atherogenesis. *Proc Natl Acad Sci U S A* 2003;100:1238-43.
12. Ferretti A, Nelson GJ, Schmidt PC, Kelley DS, Bartolini G, Flanagan VP. Increased dietary arachidonic acid enhances the synthesis of vasoactive eicosanoids in humans. *Lipids* 1997;32:435-9.
13. Kelley DS, Taylor PC, Nelson GJ, Mackey BE. Arachidonic acid supplementation enhances synthesis of eicosanoids without suppressing immune functions in young healthy men. *Lipids* 1998;33:125-30.
14. Lee TH, Hoover RL, Williams JD, et al. Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on in vitro neutrophil and monocyte leukotriene generation and neutrophil function. *N Engl J Med* 1985;312:1217-24.
15. Dwyer JH, Navab M, Dwyer KM, et al. Oxygenated carotenoid lutein and progression of early atherosclerosis: the Los Angeles Atherosclerosis Study. *Circulation* 2001;103:2922-7.
16. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK Jr. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. *N Engl J Med* 1999;340:14-22.
17. Sun P, Dwyer KM, Merz CN, et al. Blood pressure, LDL cholesterol, and intima-media thickness: a test of the "response to injury" hypothesis of atherosclerosis. *Arterioscler Thromb Vasc Biol* 2000;20:2005-10.
18. Dwyer JH, Sun P, Kwong-Fu H, Dwyer KM, Selzer RH. Automated intima-media thickness: the Los Angeles Atherosclerosis Study. *Ultrasound Med Biol* 1998;24:981-7.
19. Feskanich D, Sielaff BH, Chong K, Buzzard IM. Computerized collection and analysis of dietary intake information. *Comput Methods Programs Biomed* 1989;30:47-57.
20. Cantwell MM. Assessment of individual fatty acid intake. *Proc Nutr Soc* 2000;59:187-91.
21. Andreotti F, Burzotta F, Mazza A, Manzoli A, Robinson K, Maseri A. Homocysteine and arterial occlusive disease: a concise review. *Cardiologia* 1999;44:341-5.
22. Boekholdt SM, Bijsterveld NR, Moons AHM, Levi M, Buller HR, Peters RJG. Genetic variation in coagulation and fibrinolytic proteins and their relation with acute myocardial infarction: a systematic review. *Circulation* 2001;104:3063-8.
23. Zee RYL, Ridker PM. Polymorphism in the human C-reactive protein (CRP) gene, plasma concentrations of CRP, and the risk of future arterial thrombosis. *Atherosclerosis* 2002;162:217-9.
24. Kiechl S, Lorenz E, Reindl M, et al. Toll-like receptor 4 polymorphisms and atherogenesis. *N Engl J Med* 2002;347:185-92.
25. Lotzer K, Spanbroek R, Hildner M, et al. Differential leukotriene receptor expression and calcium responses in endothelial cells and macrophages indicate 5-lipoxygenase-dependent circuits of inflammation and atherogenesis. *Arterioscler Thromb Vasc Biol* 2003;23:E32-E36.
26. Sperling RI, Benincaso AI, Knoell CT, Larkin JK, Austen KE, Robinson DR. Dietary omega-3 polyunsaturated fatty acids inhibit phosphoinositide formation and chemotaxis in neutrophils. *J Clin Invest* 1993;91:651-60.
27. Kelley DS, Taylor PC, Nelson GJ, et al. Docosahexaenoic acid ingestion inhibits natural killer cell activity and production of inflammatory mediators in young healthy men. *Lipids* 1999;34:317-24.
28. Lee TH, Sethi T, Crea AE, et al. Characterization of leukotriene B3: comparison of its biological activities with leukotriene B4 and leukotriene B5 in complement receptor enhancement, lysozyme release and chemotaxis of human neutrophils. *Clin Sci (Lond)* 1988;74:467-75.
29. Koller M, Senkal M, Kemen M, Konig W, Zumtobel V, Muhr G. Impact of omega-3 fatty acid enriched TPN on leukotriene synthesis by leukocytes after major surgery. *Clin Nutr* 2003;22:59-64.
30. Hong S, Gronert K, Devchand PR, Mousignac RL, Serhan CN. Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood, and glial cells: autacoids in anti-inflammation. *J Biol Chem* 2003;278:14677-87.
31. Marchioli R, Barzi F, Bomba E, et al. Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. *Circulation* 2002;105:1897-903.
32. Albert CM, Campos H, Stampfer MJ, et al. Blood levels of long-chain n-3 fatty acids and the risk of sudden death. *N Engl J Med* 2002;346:1113-8.
33. Allen S, Dashwood M, Morrison K, Yacoub M. Differential leukotriene constrictor responses in human atherosclerotic coronary arteries. *Circulation* 1998;97:2406-13.
34. Prescott SM, McIntyre TM, Zimmerman GA, Stafforini DM. Sol Sherry lecture in thrombosis: molecular events in acute inflammation. *Arterioscler Thromb Vasc Biol* 2002;22:727-33.
35. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation* 2002;105:1135-43.
36. Sjostrom M, Johansson AS, Schroder O, Qiu H, Palmblad J, Haeggstrom JZ. Dominant expression of the CysLT2 receptor accounts for calcium signaling by cysteinyl leukotrienes in human umbilical vein endothelial cells. *Arterioscler Thromb Vasc Biol* 2003;23:E37-E41.
37. Silverman ES, Du J, De Sanctis GT, et al. Egr-1 and Sp1 interact functionally with the 5-lipoxygenase promoter and its naturally occurring mutants. *Am J Respir Cell Mol Biol* 1998;19:316-23.
38. Uhl J, Klan N, Rose M, Entian KD, Wertz O, Steinhilber D. The 5-lipoxygenase promoter is regulated by DNA methylation. *J Biol Chem* 2002;277:4374-9.
39. Serio KJ, Hodulik CR, Bigby TD. Sp1 and Sp3 function as key regulators of leukotriene C(4) synthase gene expression in the monocyte-like cell line, THP-1. *Am J Respir Cell Mol Biol* 2000;23:234-40.
40. Sampson AP, Siddiqui S, Buchanan D, et al. Variant LTC(4) synthase allele modifies cysteinyl leukotriene synthesis in eosinophils and predicts clinical response to zafirlukast. *Thorax* 2000;55:Suppl 2:S28-S31.
41. Serhan CN. Lipoxins and novel aspirin-triggered 15-epi-lipoxins (ATL): a jungle of cell-cell interactions or a therapeutic opportunity? *Prostaglandins* 1997;53:107-37.
42. Goulet JL, Snouwaert JN, Latour AM, Coffman TM, Koller BH. Altered inflammatory responses in leukotriene-deficient mice. *Proc Natl Acad Sci U S A* 1994;91:12852-6.
43. Cyrus T, Pratico D, Zhao L, et al. Absence of 12/15-lipoxygenase expression decreases lipid peroxidation and atherogenesis in apolipoprotein e-deficient mice. *Circulation* 2001;103:2277-82.
44. Burleigh ME, Babaev VR, Oates JA, et al. Cyclooxygenase-2 promotes early atherosclerotic lesion formation in LDL receptor-deficient mice. *Circulation* 2002;105:1816-23.
45. Li B, Birdwell C, Whelan J. Antithetic relationship of dietary arachidonic acid and eicosapentaenoic acid on eicosanoid production in vivo. *J Lipid Res* 1994;35:1869-77.
46. Shen J, Herderick E, Cornhill JF, et al. Macrophage-mediated 15-lipoxygenase expression protects against atherosclerosis development. *J Clin Invest* 1996;98:2201-8.
47. Cayatte AJ, Du Y, Oliver-Krasinski J, Lavielle G, Verbeuren TJ, Cohen RA. The thromboxane receptor antagonist S18886 but not aspirin inhibits atherogenesis in apo E-deficient mice: evidence that eicosanoids other than thromboxane contribute to atherosclerosis. *Arterioscler Thromb Vasc Biol* 2000;20:1724-8.
48. Serhan CN, Takano T, Chiang N, Gronert K, Clish CB. Formation of endogenous "antiinflammatory" lipid mediators by transcellular biosynthesis: lipoxins and aspirin-triggered lipoxins inhibit neutrophil recruitment and vascular permeability. *Am J Respir Crit Care Med* 2000;161:S95-S101.
49. Radmark O. 5-Lipoxygenase-derived leukotrienes: mediators also of atherosclerotic inflammation. *Arterioscler Thromb Vasc Biol* 2003;23:1140-2.

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