Nutritional mechanisms that influence cardiovascular disease[1–4]

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ABSTRACT

Current evidence suggests that most significant risk factors for heart disease have been identified. Although age, sex, and genetics are important unmodifiable risk factors, most new cases of acute myocardial infarctions today can be predicted by the presence and level of 9 risk (or cardioprotective) factors that can easily be assessed and, most importantly, modified. These risk factors are the same in almost every geographic region and in every racial/ethnic group worldwide and are consistent in men and women. Eight of these 9 risk factors are influenced by diet, and most act by promoting atherogenesis, which is the most important background condition for cardiovascular disease. Dietary interventions mostly affect atherogenesis by modulating, at the cellular level, proinflammatory processes that initiate and perpetuate endothelial dysfunction, plaque formation, and, eventually, plaque rupture. For example, there is now enough evidence, both epidemiologic and clinical, of the beneficial effects of n−3 fatty acids. Either as part of a normal low-fat diet or as supplements, these fatty acids are now recommended to prevent cardiovascular disease. This review will summarize the mechanisms by which diet may influence atherogenesis through the early inception, progression, and clinical emergence of atherosclerosis, with a special focus on n−3 fatty acids. *Am J Clin Nutr* 2006;83(suppl):421S–6S.

KEY WORDS Atherosclerosis, diabetes, endothelial dysfunction, insulin resistance, obesity, omega-3 fatty acids, n−3 fatty acids, oleate, docosahexaenoic acid, eicosapentaenoic acid, arachidonic acid, 5-lipoxygenase polymorphisms, leukotrienes

INTRODUCTION

Cardiovascular disease is likely the most studied chronic human disease, yet it remains the most common throughout the world and is the leading cause of premature morbidity and mortality in industrialized countries (1). Epidemiologic research has identified several risk factors, but the extent to which these can account for cardiovascular disease has been largely unknown until recently. It was actually estimated that known risk factors account for no more than 50% of cardiovascular disease risk, leaving much of the variability of our estimates largely unexplained. However, the recently reported INTERHEART study (2) challenged the current wisdom with respect to the extent that risk factors explain the general risk of cardiovascular disease in the population.

INTERHEART was a standardized case-control study of factors associated with the first occurrence of acute myocardial infarction in 52 countries around the world and compared 15 152 cases with 14 820 controls with no history of heart disease (2). The risk factors evaluated were all modifiable and included smoking, history of hypertension and diabetes mellitus, waist-to-hip ratio, dietary patterns, physical activity, alcohol consumption, the ratio of apolipoprotein (apo) B to apo A-I (which roughly corresponds to the ratio of LDL to HDL cholesterol), and psychosocial factors. Odds ratios (ORs) were calculated, as was the population-attributable risk (the latter of which takes into account both the OR and the prevalence of the risk factor in the population and indicates the percentage of risk that can be attributed to that specific risk factor or combination of risk factors). Collectively, the 9 risk factors accounted for 90% of the risk of a first occurrence of acute myocardial infarction in men and 94% of the risk in women. After adjustment for age, sex, and the above 9 risk factors, the risk of acute myocardial infarction for persons with all 9 risk factors combined, compared with those with no risk factors, was considerably higher (OR: 129.20; 99% CI: 90.24, 184.99). When the model included only those in the extremes (ie, the top versus lowest tertile), the OR increased even more drastically (OR: 333.7; 99% CI: 230.2, 483.9). Globally, 80% of the occurrence of a first acute myocardial infarction was predicted by the combination of smoking, dyslipidemia, hypertension, diabetes, and obesity.

Some of the variables considered were protective (or "negative") risk factors. Their presence significantly decreased the risk. For example, adding exercise, dietary changes, and moderate alcohol consumption to persons with an otherwise high-risk profile incrementally downgraded the overall risk of a first acute myocardial infarction. The impact of INTERHEART is not so much in the identification of new risk factors for cardiovascular disease (all factors considered have indeed been known for a long time), but in the strength of the association between risk factors and the occurrence of a first acute myocardial infarction and in the notion that all 9 identified risk factors are modifiable. Noticeably, 8 of 9 of these factors are strongly influenced and

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modifiable by diet. We now clearly know how ample the opportunities are for prevention through changes in dietary and lifestyle patterns.

MECHANISMS OF CARDIOVASCULAR DISEASE: ATHEROSCLEROSIS

Atherosclerosis is by far the most important underlying pathologic process for cardiovascular disease. Therefore, an understanding of the processes involved in the initiation and progression of atherosclerosis, ending in the rupture of the atherosclerotic plaque and the formation of a thrombus, is intimately entwined in the mechanisms by which nutritional intake influences cardiovascular disease.

The initiating event in atherosclerosis involves the induction of endothelial dysfunction by atherogenic triggers, the best recognized of which are modified or oxidized LDL and the advanced glycation end products that occur in diabetes. This functional alteration in turn causes the increased expression of atherogenic signal molecules, including adhesion molecules, such as vascular cell adhesion molecule 1 (VCAM-1); chemoattractants, such as monocyte chemoattractant protein 1 (MCP-1); and a host of growth factors and cytokines, among which are macrophage colony stimulating factor, CD40 ligand (CD154), interferon γ, tumor necrosis factor α (TNF-α), interleukin 1 (IL-1), and IL-6 (3–5). These signaling molecules allow the adhesion of monocytes and T lymphocytes to the arterial endothelium and their penetration into the intima. Monocytes undergo transformation and replication into macrophages and ultimately form the lipid-rich foam cells that are characteristic of the fatty streak, the first morphologically recognizable precursor of the atherosclerotic plaque. The same signal molecules are also responsible for the growth and the eventual destabilization of the plaque, being able to promote plaque rupture and the thrombogenic nature of the plaque content itself through the increased expression of molecules such as tissue factor (4, 6).

The above-listed signal molecules are mostly products of activated macrophages, lymphocytes, and endothelial cells, and they are inducible by cytokines (3, 4, 7, 8). Therefore, they can be considered secondary mediators of local vascular inflammation. Resident macrophages perpetuate the cycle of local inflammation by releasing inflammatory cytokines, which result in the production of matrix-degrading metalloproteinases that contribute to plaque rupture, and tissue factor, which increases plaque thrombogenicity (6, 8).

HOW NUTRITION INFLUENCES CARDIOVASCULAR DISEASE RISK

Effect of energy intake

Obesity, particularly central obesity, is a recognized risk factor for cardiovascular disease and is the consequence of a positive energy balance. The mechanisms responsible for this increased risk appear to be related to obesity itself and to a condition closely associated with it—insulin resistance. Insulin resistance, which is defined as a decreased response of peripheral tissues to insulin action, predisposes individuals to developing type 2 diabetes.

Several studies have linked insulin resistance to systemic inflammation (9, 10), possibly as the result of increased concentrations of circulating free fatty acids (11, 12). When hyperglycemia develops in type 2 diabetes, it produces toxic effects on the endothelium both directly (13, 14) and through the formation of advanced glycation end products (15–17), which can themselves perpetuate an inflammatory response in the endothelium. However, macrovasculopathy (atherosclerosis) may precede by as many as 10 y the development of diabetes (18). This leads to the idea that insulin itself can be a proatherogenic hormone (19–21). Evidence now exists that insulin allows the initiation and perpetuation of vascular inflammation, through the increased gene expression of VCAM-1, MCP-1, macrophage colony stimulating factor, CD-40L, and similar molecules (21). Therefore, the increase in insulin production and plasma concentration that accompanies the compensated phase of insulin resistance appears to increase atherogenic risk directly.

Obesity not only predisposes individuals to insulin resistance and type 2 diabetes, but it is also associated with a chronic inflammatory response in itself (22). There is indeed evidence that obesity is associated with macrophage accumulation in the adipose tissue (23, 24). One recent study showed that the degree of infiltration of the adipose tissue by activated macrophages closely correlates with the adipocyte area in mice and that adipose-tissue-associated macrophage number is directly proportional to adiposity both in mice and in humans (23). Another study reported that many inflammatory and macrophage-specific genes are dramatically upregulated in white adipose tissue in mouse models of genetic obesity and obesity induced by a high-fat diet (24).

It thus appears that obesity increases cardiovascular disease risk through 2 inflammation-mediated pathways: it promotes insulin resistance, which increases the expression of many inflammatory mediators, and it is directly associated with an inflammatory response itself, which affects the production of several proinflammatory cytokines (adipokines) and hormones. If a positive energy balance is proinflammatory and increases cardiovascular disease risk, the reverse is also true: caloric restriction reduces inflammation in parallel to—and likely preceding—the reduction of cardiovascular disease risk. The effect of caloric restriction protocols on risk factors for atherosclerosis has been assessed comparing individuals who had been restricting their food intake for 6 y and age-matched healthy individuals consuming a typical American diet (25). The caloric restriction group had significantly lower body mass indexes and percentages of body fat than did the group consuming the American diet. Total serum cholesterol concentrations, LDL-cholesterol concentrations, the ratio of serum total cholesterol to HDL cholesterol, triacylglycerol concentrations, fasting glucose, fasting insulin, C-reactive protein (CRP), platelet-derived growth factors A and B, and systolic and diastolic blood pressure were all markedly lower, and HDL cholesterol was higher, in the calorie restriction group than in the American diet group (25). Carotid artery intima-media thickness, which is an indicator of the total atherosclerotic burden, was $\approx$40% less in the calorie restriction group than in the American diet group, which supports the concept that long-term caloric restriction has a powerful protective effect against atherosclerosis (25). None of the individuals in the caloric restriction group had evidence of atherosclerotic plaques, which is defined as a carotid intima-media thickness of $>$1.0 mm and an increase of $\geq$100% compared with an adjacent wall segment.

The extremely low CRP concentrations observed in the calorie restriction group in the above-referenced study provide preliminary evidence that caloric restriction also reduces inflammation. CRP is a marker of systemic inflammation and may also play a

role itself in the inflammatory process, which would explain why it is such a consistent risk factor for cardiovascular events (26–28). Very low plasma insulin concentrations and serum-derived platelet-derived growth factor A and B concentrations in the calorie restriction group suggest that caloric restriction also results in a decreased stimulus for cell proliferation.

Effects of n−3 polyunsaturated fatty acids

Clear evidence exists showing that serum cholesterol correlates with the risk of coronary artery disease, myocardial infarction, and coronary death (29). This can also be expressed by the relation between the percentage of daily energy intake from saturated fats, which correlates closely with serum cholesterol concentrations, and the degree of coronary artery disease. Although serum cholesterol is a strong correlate of coronary artery disease, the slope of the relation differs in different populations and within study subgroups, which suggests that there is a large potential for additional modulators of disease risk. Several studies have addressed the relation between the intake of n−3 polyunsaturated fatty acids (PUFAs) and cardiovascular disease and the effect of increased n−3 PUFA intake on cardiovascular events (30–35).

The Lyon Diet Heart Study (34) was a randomized secondary prevention trial testing whether a Mediterranean-type diet, which is characterized by low intakes of total and saturated fats and increased intakes of marine or plant-derived n−3 PUFAs, fresh fruit and vegetables, legumes, high-fiber cereals, antioxidants, minerals, vegetable proteins, and B vitamins, might reduce the recurrence rate for individuals who had already experienced a myocardial infarction compared with the rate for persons consuming a prudent American diet. The study also examined the relation between traditional cardiovascular disease risk factors, dietary patterns, and the occurrence of complications. An intermediate analysis showed a striking protective effect of the Mediterranean diet: a 73% reduction in the risk of new major cardiac events. All-cause and cardiovascular mortality and the combination of recurrent myocardial infarction and cardiac death were lower in the Mediterranean diet group than in the American diet group. The protective effect was maintained up to 4 y after the first infarction, thus confirming the intermediate analyses (36). Major traditional risk factors, such as high serum cholesterol and blood pressure, were shown to be independent predictors of the recurrence of myocardial infarction, which indicates that the Mediterranean diet did not alter, at least not qualitatively, the usual relations between major risk factors and the recurrence of infarction. This trial clearly showed that a comprehensive strategy to decrease cardiovascular morbidity and mortality should include a cardioprotective diet.

The low mortality from cardiovascular disease among traditionally living Eskimos and Alaskan Natives has been attributed to less atherosclerosis in the coronary arteries and elsewhere than in non-natives because of a high dietary intake of n−3 PUFAs from fish or fish-derived products (37). Newman et al (32) evaluated the extent of atherosclerotic lesions in the coronary arteries and the aortas of Alaskan Natives and of non-natives to test the hypothesis of differences in atherosclerosis extension and severity. Standardized comparisons between samples from 103 Native and 101 non-native residents showed that the extent of fatty streaks and raised plaques increased with age in both groups, but the prevalence of these lesions in Natives was consistently and significantly lower than in non-natives (**Figure 1**). Interestingly,

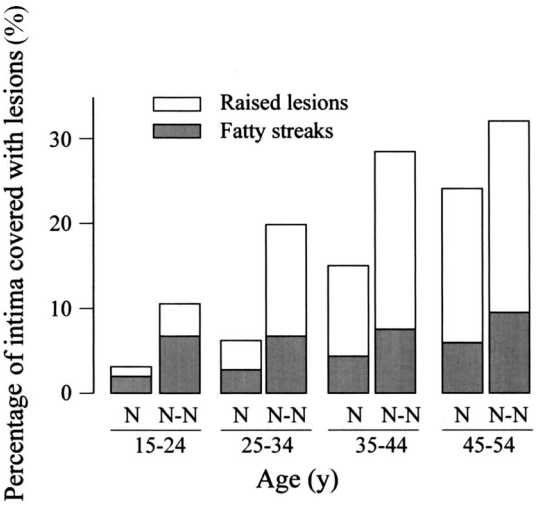

FIGURE 1. Percentage of coverage of the aorta with fatty streaks and raised plaques in Alaskan natives (N) and non-natives (N-N), divided by age. Notice the larger difference, attributable to the prevalence of fatty streaks, in the younger age groups. Data are from reference 32.

there was an even more striking difference in the percentage of aorta covered with fatty streaks, which are the precursors of atherosclerotic plaques, in younger individuals (32), which suggests some protection from the early inception of atherosclerosis. The differences in cardiovascular mortality between Alaskan Natives and non-natives thus appear to be, at least in part, the result of less atherosclerosis in the Native population.

Hu et al (31) prospectively examined the association between intake of fish and n−3 PUFAs and the risk of coronary heart disease and total mortality among 5103 female nurses with diagnosed type 2 diabetes but free of cardiovascular disease or cancer at baseline. Between 1980 and 1996 (45 845 person-years of follow-up), 362 incident cases of coronary heart disease (141 coronary heart disease deaths and 221 nonfatal myocardial infarctions) and 468 deaths from all causes were documented. After adjustment for age, smoking, and other established coronary risk factors, the relative risk of coronary heart disease in women who seldom consumed fish was compared with the risk in those who consumed variable amounts of fish. Higher consumption of fish was associated with significantly lower total mortality, whereas higher consumption of long-chain n−3 PUFAs was associated with a trend toward lower incidence of coronary heart disease and total mortality (31). These findings suggest that regular fish consumption should be considered as part of a healthy diet for the management of diabetes and that this dietary protection likely occurs through the increased nutritional intake of n−3 PUFAs.

There are indications that other minor dietary components in Mediterranean diets, from olive oil or wine, also have antiatherogenic effects. Natural olive oil and red wine polyphenols, including elenolic acids, tyrosol, oleuropein glycoside, hydroxytyrosol, *trans*-resveratrol, and oleuropein aglycone, collaborate in decreasing VCAM-1 expression and cytokine production in endothelial cells, likely through a reduced activation of nuclear factor-κB (NF-κB), a transcription factor that is central to the mechanisms of vascular inflammation (38).

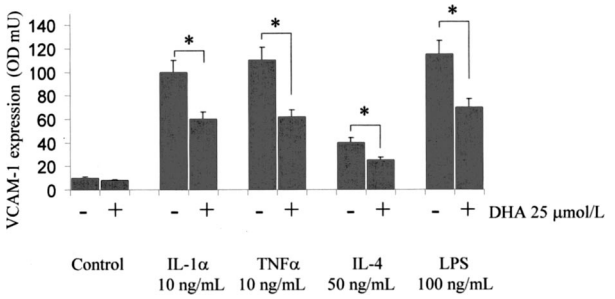

FIGURE 2. The inhibition of vascular cell adhesion molecule 1 (VCAM-1) surface expression by docosahexaenoic acid (DHA) occurring with diverse stimuli, including interleukin 1α (IL-1α), tumor necrosis factor α (TNF-α), IL-4, and bacterial lipopolysaccharide (LPS). Values are means ($\pm$SEMs). OD, optical density. *Significant difference, $P < 0.01$.

Molecular mechanisms by which n−3 PUFAs affect atherosclerosis

The mechanisms underlying the benefits of n−3 PUFAs on atherosclerosis have been the object of much laboratory and clinical research. The emerging concept is that these nutrients can modulate the intimate mechanisms of development and progression of atherosclerosis. One study, from our group, investigated the intracellular generation of reactive oxygen species (ROS) in endothelial cells treated with various fatty acids (39). It is commonly accepted that ROS, generated in low concentrations, may function intracellularly as second messengers in mediating the activation of NF-κB and activator protein 1 (40–42). In our study, endothelial cells were stimulated with cytokines or lipopolysaccharide. Preincubation of endothelial cells with the monounsaturated fatty acid oleate blunted the endothelial response to the proinflammatory stimulation in terms of VCAM-1 surface expression or the release of macrophage colony stimulating factor. Such effects were accompanied by a decreased production of ROS and a sparing in the depletion of the intracellular antioxidant reduced glutathione. This occurred without any change in the activity of the glutathione-related antioxidant enzymes superoxide dismutase and catalase (39). All such effects were magnified when n−3 PUFAs, such as docosahexaenoic acid (DHA), were used instead of oleate. Because these fatty acids also apparently compete for different intracellular phospholipid pools, the effects of oleate and DHA can be additive (5, 39). These results indicate that oleate and n−3 PUFAs may exert a direct vascular atheroprotective effect by inhibiting endothelial activation through a quenching of stimuli-induced increase in ROS.

Results of other experiments showed that the in vitro expression of VCAM-1 by endothelial cells stimulated with inflammatory cytokines, such as IL-4, IL-1, and TNF-α, decreases in a time- and concentration-dependent manner if the cells are preincubated with DHA (43; **Figure 2**). Interestingly, another n−3 PUFA, eicosapentaenoic acid (EPA), has a smaller effect (44). Pretreatment with DHA also reduces the adhesion of human monocytes and of monocytic U937 cells to the endothelial cells in response to stimulation (44).

It therefore appears that there is a down-regulation of the intracellular mechanisms that lead to the expression of proatherogenic genes. In this case, the final mechanism is the activation of the transcription factor NF-κB, which regulates the production of many soluble cytokines from endothelial cells and

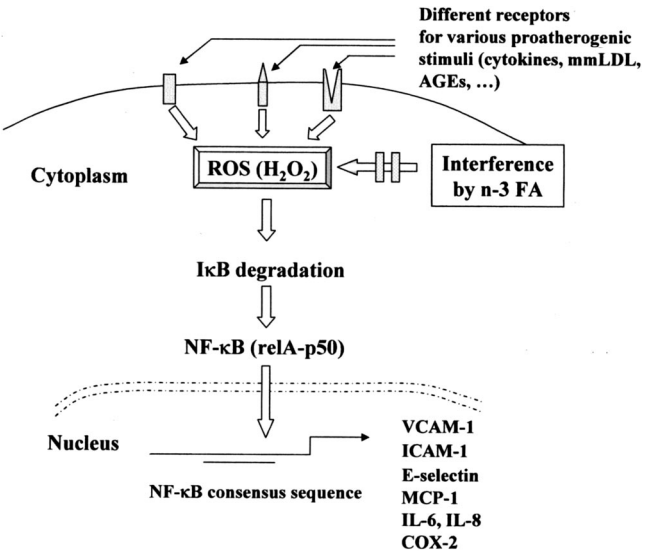

FIGURE 3. A scheme showing the putative site of action of n−3 fatty acids (FA) on endothelial activation at the level of generation of reactive oxygen species (ROS). AGEs, advanced glycation end products; COX, cyclooxygenase; ICAM-1, intercellular adhesion molecule 1; IκB, NF-κB inhibitor; IL, interleukin; MCP-1, monocyte chemoattractant protein 1; mmLDL, minimally modified LDL; NF-κB, nuclear factor κB; VCAM-1, vascular cell adhesion molecule 1.

macrophages and the expression of adhesion molecules in response to inflammatory cytokines. The interference by DHA upstream of NF-κB appears to be at the level of intracellular production of ROS, which are in turn able to activate the transcription factor NF-κB (**Figure 3**). This may be a general explanation of why the risk of cardiovascular disease in many populations is inversely related to the dietary intake of n−3 PUFAs (5).

GENETIC RISK OF CARDIOVASCULAR DISEASE AND ITS MODULATION BY NUTRIENTS

Arachidonic acid, a fatty acid present in phospholipids, is a substrate for 5-lipoxygenase in white blood cells and other bone-marrow-derived cells. The enzyme 5-lipoxygenase catalyzes the conversion of arachidonic acid into leukotriene A_4, which is then in turn enzymatically converted into either leukotriene B_4 or the cysteinyl leukotrienes. Leukotriene B_4 is a potent nonspecific chemoattractant for inflammatory cells and induces the chemokinesis and adhesion of these cells to the vascular endothelium. Cysteinyl leukotrienes can increase vascular permeability. Leukotrienes may be involved in atherosclerosis (**Figure 4**; 8), and there are clues that genetic variants of 5-lipoxygenase are related to susceptibility to cardiovascular disease. Indeed, individuals carrying deletions or insertions in a region of the promoter of the 5-lipoxygenase gene exhibit increased atherosclerosis in the carotid artery, and this risk can be modulated by diet. In a randomly sampled cohort of 470 healthy middle-aged women and men from the Los Angeles Atherosclerosis Study, mean carotid intima-media thickness, adjusted for age, sex, height, and racial or ethnic group, was higher by 80 $\pm$ 19 μm among persons with the variant genotype than in carriers of the common (wild-type) allele (45). The increase in carotid artery intima-media thickness among persons with the variant genotype was similar in this cohort to that associated with diabetes, the strongest common

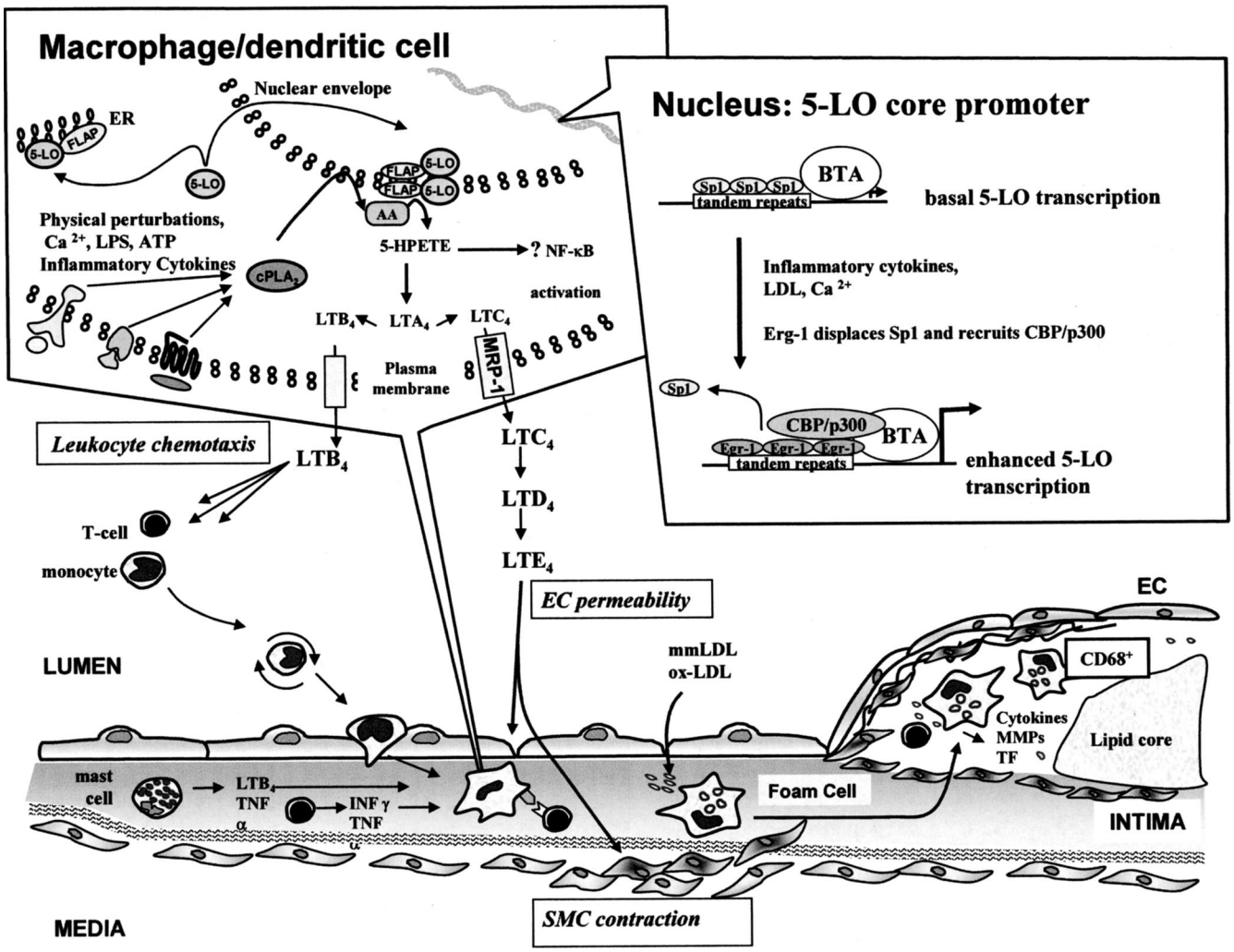

FIGURE 4. The main roles of 5-lipoxygenase (5-LO) in atherosclerosis. Early development of lesions is caused by invasion of the intima by monocytes, followed by the transformation of monocyte-derived macrophages into foam cells through the uptake of minimally modified (mm) or oxidized (ox) LDL. Leukotrienes (LTs) may contribute to atherosclerosis by promoting nonspecific leukocyte chemotaxis (LTB_4) and by increasing vascular permeability (cysteinyl leukotrienes). The activation and gene expression of 5-LO, the enzyme responsible for the initiation of the leukotriene biosynthetic pathway from arachidonic acid (AA), can be increased by various cytokines in inflammatory conditions. Resident macrophages perpetuate a vicious circle of local inflammation by releasing inflammatory cytokines, matrix-degrading metalloproteinases (contributing to plaque rupture), and tissue factor (TF; increasing plaque thrombogenicity), as well as by producing more LTs. The inset to the right shows the structure and mode of action of the 5-LO core promoter, where interplay of various transcription factors (including sp-1, egr-1, and CBP/p300) determine the transcription rate of the enzyme. Mutations in the 5-LO core promoter alter the rate of transcription of the enzyme, a process that can be modulated by the availability of the substrate AA through the diet and by competition with n−3 polyunsaturated fatty acids. *See* also reference 8. BTA, baseline transcriptional activator; EC, endothelial cell; ER, endoplasmic reticulum; FLAP, 5-lipoxygenase-activating protein; 5-HPETE, 5-hydroperoxy-eicosatetraenoic acid; INFγ, interferon γ; LPS, lipopolysaccharide; MMP, matrix metalloproteinase; MRP-1, membrane relay protein 1; NF-κB, nuclear factor-κB; $cPLA_2$, cellular phospholipase A_2; SMC, smooth muscle cell; TNF, tumor necrosis factor.

cardiovascular disease risk factor. Plasma concentrations of CRP were higher by a factor of 2 among individuals with the variant genotype than in carriers of the common allele.

The atherosclerotic risk in individuals carrying the proatherogenic variants of the 5-lipoxygenase gene is a function of dietary arachidonic acid intake, and progressively increases across tertiles of arachidonic acid intake, as measured by 24-h recalls of food intake. This is compatible with the biological notion that the higher the arachidonic acid intake with the diet, the higher the production of leukotrienes. Thus, increased dietary arachidonic acid significantly enhances the proatherogenic effect of the variant genotype. Conversely, increased dietary intake of n−3 PUFAs (mostly EPA and DHA) blunts such an effect. In patients with the highest tertile of n−3 PUFA intake, there was actually no difference in intima-media thickness in patients carrying the variant alleles compared with carriers of the wild-type allele. The observed diet-gene interaction is a further example of how dietary nutrients are a potent environmental factor allowing or denying the manifestations of a specific genotype.

CONCLUSIONS

Diet can affect the vast majority of modifiable risk factors for cardiovascular disease, which are now identified as explaining a very large part of the variability in the occurrence of a first acute myocardial infarction. Diet offers incredible opportunities for prevention of cardiovascular disease. Modulation of vascular inflammation is likely the most relevant common pathogenetic

step by which nutritional factors influence cardiovascular disease. Vascular inflammation is influenced by excess caloric intake (obesity, insulin resistance), alcohol, several vitamins, dietary antioxidants, and n−3 PUFAs. Implementing relevant and successful dietary changes is the greatest challenge for preventive cardiovascular medicine after the turn of the century. A more complete understanding of how dietary changes may work will likely lead to a beneficial exploitation of such current knowledge.

RDC conceived and wrote the largest part of this review. AZ reviewed and wrote the section on leukotriene biology. SDT reviewed and wrote the section on mechanisms of n−3 fatty acids with regard to atherosclerosis. RM reviewed and wrote the section on the general regulation of gene expression by nutrients. MM reviewed and wrote the section on the intracellular quenching of reactive oxygen species by polyunsaturated fatty acids.

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