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A Dietary Mixture Containing Fish Oil, Resveratrol, Lycopene, Catechins, and Vitamins E and C Reduces Atherosclerosis in Transgenic Mice[1–3]

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Abstract

Chronic inflammation and proatherogenic lipids are important risk factors of cardiovascular disease (CVD). Specific dietary constituents such as polyphenols and fish oils may improve cardiovascular risk factors and may have a beneficial effect on disease outcomes. We hypothesized that the intake of an antiinflammatory dietary mixture (AIDM) containing resveratrol, lycopene, catechin, vitamins E and C, and fish oil would reduce inflammatory risk factors, proatherogenic lipids, and endpoint atherosclerosis. AIDM was evaluated in an inflammation model, male human C-reactive protein (CRP) transgenic mice, and an atherosclerosis model, female ApoE*3Leiden transgenic mice. Two groups of male human-CRP transgenic mice were fed AIDM [0.567% (wt:wt) powder and 0.933% (wt:wt oil)] or placebo for 6 wk. The effects of AIDM on basal and IL-1β–stimulated CRP expression were investigated. AIDM reduced cytokine-induced human CRP and fibrinogen expression in human-CRP transgenic mice. In the atherosclerosis study, 2 groups of female ApoE*3Leiden transgenic mice were fed an atherogenic diet supplemented with AIDM [0.567% (wt:wt) powder and 0.933% (wt:wt oil)] or placebo for 16 wk. AIDM strongly reduced plasma cholesterol, TG, and serum amyloid A concentrations compared with placebo. Importantly, long-term treatment of ApoE*3Leiden mice with AIDM markedly reduced the development of atherosclerosis by 96% compared with placebo. The effect on atherosclerosis was paralleled by a reduced expression of the vascular inflammation markers and adhesion molecules inter-cellular adhesion molecule-1 and E-selectin. Dietary supplementation of AIDM improves lipid and inflammatory risk factors of CVD and strongly reduces atherosclerotic lesion development in female transgenic mice. J. Nutr. 141: 863–869, 2011.

Introduction

Cardiovascular disease (CVD)[7] remains the leading cause of morbidity and mortality in the Western world. A sedentary lifestyle and Western dietary habits can contribute to an increased risk of developing CVD (1,2). For example, the consumption of diets rich in saturated fat is positively associated with elevated plasma lipid levels and a state of subacute chronic inflammation, which are 2 important risk factors promoting the onset and development of CVD (3,4). More specifically, LDL-cholesterol, TG, and the inflammatory molecules C-reactive protein (CRP), serum amyloid A (SAA), E-selectin, and inter-cellular adhesion molecule-1 (ICAM-1) are risk factors implicated in the processes leading to atherosclerosis and the occurrence of cardiovascular events (5–7).

An effective way to diminish the risk of CVD is to reduce causative risk factors. Improvement of lifestyle and dietary habits helps to reduce some of the risk factors, although the absolute effectiveness of lifestyle interventions remains questionable (8), the more so because long-lasting adjustments of lifestyle habits have proven to be difficult to implement (9). Supplementation of diets with specific protective components may be an attractive and more feasible alternative to diminish CVD. A number of dietary compounds have been associated

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[3] Supplemental Table 1 is available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at jn.nutrition.org.
[7] Abbreviations used: AIDM, antiinflammatory dietary mixture; C/EBP, CCAAT/enhancer binding protein-β; CRP, C-reactive protein; CVD, cardiovascular disease; HC diet, high-cholesterol atherogenic diet; ICAM, inter-cellular adhesion molecule; PDGF, platelet derived growth factor; SAA, serum amyloid A; STAT3, signal transducer and activator of transcription 3.
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with a reduction of primary cardiovascular risk factors and studies suggest a beneficial effect on the cardiovascular event rate in humans (10–13). For example, dietary supplementation of lycopene (60 mg/d) to men for a 3-mo period resulted in a significant 14% reduction of LDL-cholesterol (12). Increased consumption of green tea extracts rich in catechins was associated with decreased plasma cholesterol and TG (10). Also, polyphenols such as resveratrol can promote vasorelaxation and thereby protect the human endothelium (11). In addition, a recent meta-analysis showed that increased consumption of the PUFA, EPA and DHA, is linked to a reduction of plasma lipids, inflammatory mediators, and soluble adhesion molecules (13). Collectively, these studies demonstrate that intake of specific dietary components may reduce proatherogenic lipids and inflammatory risk factors participating in disease, suggesting that a combination of several of these beneficial components into a mixture may be an effective strategy for disease prevention.

Recently, we tested such a mixture containing fish oil, resveratrol, lycopene, catechin, d-α-tocopherol, and vitamin C [antiinflammatory dietary mixture (AIDM)] in healthy but overweight volunteers (14). Five weeks of AIDM treatment had a positive effect on factors implicated in inflammation, oxidative stress, and dyslipidemia. Here we tested AIDM at a concentration equivalent to the dose used in the human trial in "humanized": animal models (15–17) of inflammation (human-CRP transgenic mice) and atherosclerosis (ApoE*3Leiden transgenic mice) to investigate putatively beneficial effects of AIDM on inflammatory risk factors and cardiovascular endpoints.

Methods

Mice

Animal experiments conformed to the regulations set forward by the Netherlands Law on Animal Experiments and the Institutional Animal Care and Use Committee of TNO. Mice consumed diets and water ad libitum.

All mice used are on a C57BL/6 background. Male human-CRP transgenic mice (TNO-Pharma, Gaubius Laboratory) carry a 31-kb human DNA fragment containing the human *CRP* gene, including all known *cis*-acting regulatory elements, i.e. the entire human CRP promoter, and show a human-like pattern of expression (18). Male mice were used because their baseline CRP level in plasma is sufficiently high to be detected by ELISA, whereas this is not the case in females. ApoE*3Leiden transgenic mice (TNO-Pharma, Gaubius Laboratory) were characterized for expression of human ApoE by ELISA (17). Female ApoE*3Leiden mice were used because they are more prone than their male counterparts to develop atherosclerosis (19).

AIDM

AIDM was recently tested in humans (14) and the same batch was used herein. AIDM consists of a powder and an oil and the composition is reported in (14). Briefly, the powder (Microz Food Supplements) contained 6.3 mg resveratrol, tomato extract containing 3.75 mg lycopene, 94.5 mg green tea extract (40% epigallocatechin gallate), 90.7 mg d-α-tocopherol, and 125 mg vitamin C. The oil contained 1200 mg fish oil (380 mg EPA, 260 mg DHA, and 60 mg other PUFA) (Omega-3–700, Solgar Vitamin and Herb). The powder and oil were mixed into a nonpurified diet (CRP study) or an atherogenic diet (ApoE*3Leiden studies). The placebo contained 365 mg microcrystalline cellulose (Microz Food Supplements) and 1.36 g soy lecithin (soya lecithin; Solgar Vitamin and Herb) and was also mixed into the diets.

CRP study

Two groups of male human-CRP transgenic mice (n = 7/group) were fed a standard nonpurified diet [Ssniff R/M-H Chow Diet, Spezialdiäten; crude nutrients (in g/kg dry matter): protein, 216; N-free extract, 617; fat, 38; fiber, 56; ash, 73; supplemented with vitamins and minerals; 16.0 MJ/kg metabolizable energy]. One group received AIDM [0.567% (wt:wt) powder and 0.933% (wt:wt) oil] that was mixed into the nonpurified diet. The dose of AIDM used for the CRP study was equal to the highest dose used in ApoE*3Leiden mice (see below). The other group received a similar amount of placebo, which was composed as specified above. After 6 wk of treatment, mice were stimulated with an i.p. injection of 125k IU IL-1β (Sanvertech). IL-1β induces CRP expression with maximal effect 18 h after the injection (15). Tail blood samples were collected before and 18 h after IL-1β injection with EDTA as an anticoagulant.

Determination of dose and atherosclerosis studies

Dose determination study. During a run-in period of 3 wk, ApoE*3Leiden mice (12 wk old; n = 14/group) were fed a 0.5% (wt:wt) cholesterol-containing, atherogenic, high-cholesterol diet (referred to as the HC diet; Hope Farms). This HC diet is a well-established diet to induce atherosclerosis (for diet composition, see **Supplemental Table 1**) containing (all wt:wt) 15% cacao butter, 1% corn oil, 40.5% sucrose, 20% acid casein, 10% corn starch, 5.7% cellulose, and 0.5% cholesterol (17). Mice were matched into 2 groups based on plasma cholesterol levels. One group received increasing doses of AIDM mixed into the HC diet: from wk 0 to 2, low dose [0.063% (wt:wt) powder and 0.104% (wt:wt) oil]; from wk 2 to 4, medium dose [0.189% (wt:wt) powder and 0.311% (wt:wt) oil]; and from wk 4 to 6, high dose [0.567% (wt:wt) powder and 0.933% (wt:wt) oil]. The other group consumed the HC diet containing increasing doses of placebo. Blood samples were taken at the start and end of each dosing period.

Atherosclerosis study. During a run-in period of 3 wk, 30 female E3L mice received the HC diet. Then, mice were matched into 2 groups based on plasma cholesterol and TG concentrations. One group was treated for 16 wk with HC containing the high-dose AIDM (AIDM group). The placebo group consumed the HC containing the same dose of placebo. Tail blood samples were collected at 0, 2, 4, 8, 12, and 16 wk. Mice were killed by carbon dioxide inhalation and hearts with aortic roots were collected.

Analysis of plasma lipids, lipoproteins, and inflammation markers

Plasma total cholesterol and TG concentrations were measured in blood samples collected into EDTA-tubes from mice after 4 h of food deprivation [kit nos. 11489437 and 11488872, Roche Diagnostics, respectively (20)]. For lipoprotein profiles, pooled plasma obtained during wk 16 was fractionated using an AKTA FPLC system (Pharmacia) (21). The plasma levels of SAA were determined by ELISA (Tridelta; catalog no. TP802-M) and fibrinogen was quantified with an in-house ELISA (22). E-selectin and ICAM-1 were quantified by established ELISA (R&D Systems Europe).

Atherosclerotic lesion analysis

Hearts were fixed and embedded in paraffin to prepare serial cross-sections (5 μm thick) throughout the entire aortic root area for (immuno) histological analysis (16). Cross-sections were stained with hematoxylin-phloxine-saffron and atherosclerosis was analyzed without knowledge of treatment groups. Due to a technical problem, 1 heart from the placebo group was lost. For immunostaining of ICAM-1, antibody GTX76543 from GeneTex, Biotechnology was used.

Statistical methods

Data in the CRP study were analyzed by 2-way ANOVA (CRP study: AIDM $\times$ IL-1β; dose study: treatment $\times$ dose) or repeated-measures ANOVA (atherosclerosis study). When appropriate, data were then subject to the least significant difference post hoc test. Based on Levene's test for equal variances, the nonparametric Mann-Whitney U test was used to analyze the SAA data in the atherosclerosis study. In all tests performed, the null hypothesis was rejected at the level of 5% probability (α = 0.05).

Results

AIDM reduces CRP expression in male human-CRP transgenic mice. At the start, the body weight of the placebo (31.4 $\pm$ 2.4 g) and AIDM groups (32.0 $\pm$ 2.7 g) did not differ nor did

they differ at the end of the study (31.9 ± 2.8 and 32.0 ± 3.2 g, respectively). AIDM also did not significantly affect daily food intake, which was 3.6 ± 0.5 g/d in the placebo group and 3.3 ± 0.1 g/d in the AIDM group.

The placebo and AIDM groups had comparable baseline CRP concentrations of 7.3 ± 3.4 and AIDM 6.4 ± 1.8 mg/L, respectively, which did not change in either group and were 7.1 ± 2.1 and 5.9 ± 2.1 mg/L, respectively, at the end of the study.

The i.p. injection of IL-1β resulted in a significant 3-fold stimulation of CRP (**Fig. 1***A*) in the placebo group. In the AIDM group, CRP levels also increased significantly after IL-1β injection. However, compared with placebo-treated mice, the inflammatory response of AIDM-treated mice was significantly quenched. Fibrinogen, another hepatic inflammation marker, had a similar response to CRP (Fig. 1*B*). At the start, plasma fibrinogen levels were comparable in the placebo group (2.2 ± 0.7 g/L) and the AIDM group (2.3 ± 0.9 g/L). The fibrinogen concentration did not change over time in either group (data not shown). Stimulation with IL-1β significantly increased fibrinogen expression in the placebo group and AIDM fully quenched this effect (Fig. 1*B*). Furthermore, AIDM reduced plasma TG concentrations from 1.2 ± 0.4 to 0.7 ± 0.2 mmol/L, whereas there was no change in the placebo group with concentrations of 1.3 ± 0.5 and 1.0 ± 0.3 mmol/L at the beginning and end of the study, respectively.

*Dose determination study with AIDM in female ApoE*3-Leiden mice.* To define the AIDM dose that was needed to affect cardiovascular risk factors under atherogenic conditions, a dose-finding study in ApoE*3Leiden mice was performed. At the start of the intervention, the plasma cholesterol concentration did not differ between the placebo (16.3 ± 2.0 mmol/L) and AIDM (16.4 ± 3.7 mmol/L) groups. With increasing dietary concentrations of AIMD (switch to higher dose every 2 wk), plasma cholesterol levels decreased significantly and dose dependently. The maximal plasma cholesterol-lowering effect was achieved with the highest dose of AIDM (44% reduction; 9.1 ± 1.3 mmol/L). The placebo treatment did not affect the plasma cholesterol concentrations. AIDM treatment also dose-dependently decreased plasma TG concentrations from 2.5 ± 0.7 mmol/L at baseline to 0.8 ± 0.2 mmol/L at the end of the study ($P < 0.001$). The concentration in the group given the high dose of AIDM was 48% lower than in the placebo group. The plasma TG concentration also decreased in the placebo group from 2.6 ± 0.6 mmol/L to 1.5 ± 0.2 mmol/L ($P < 0.05$), but the effect was less pronounced than in the AIDM-treated group.

To test a possible antiinflammatory effect of AIDM, we measured the plasma concentration of SAA, a systemic inflammation marker. At the start of the intervention, SAA did not differ between the placebo (8.4 ± 1.8 mg/L) and AIDM (7.0 ± 2.3 mg/L) groups. The low and medium doses of AIDM did not affect the SAA concentration. However, the high dose of AIDM resulted in a plasma SAA concentration (3.6 ± 1.7 mg/L) that was lower than in the placebo group (8.9 ± 4.0 mg/L; $P < 0.01$). Therefore, the high dose of AIMD was used in a subsequent long-term intervention study.

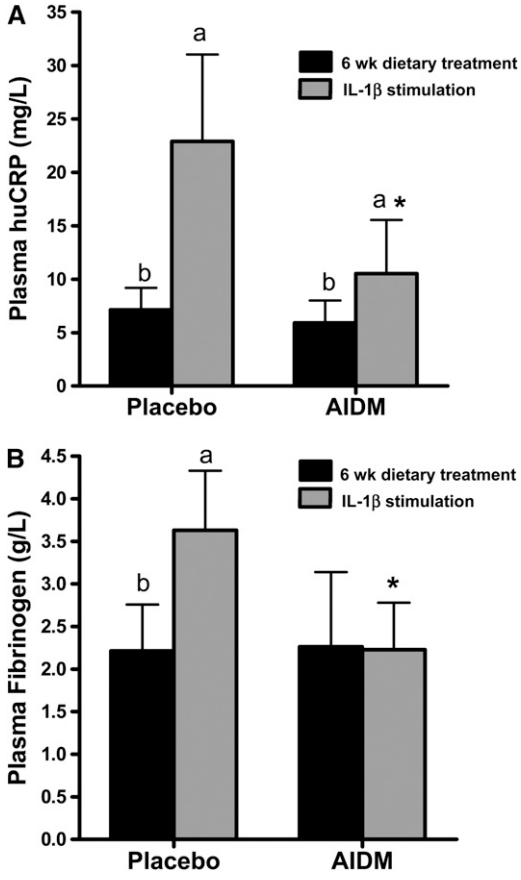

FIGURE 1 Plasma human CRP (*A*) and fibrinogen (*B*) concentrations in male human-CRP transgenic mice fed placebo or AIDM for 6 wk and before and after IL-1β stimulation. Values are mean ± SD, $n = 7$. Within a diet group, means without a common letter differ, $P < 0.05$. *Different from corresponding placebo, $P < 0.05$.

AIDM treatment attenuates the development of atherosclerosis. During the atherosclerosis study, the gain in body weight in the AIDM group (4.5 ± 2.1 g) was greater in the placebo group (2.0 ± 0.9 g; $P < 0.01$). This effect was paralleled by a greater daily food intake in the AIDM group (2.6 ± 0.1 g/d) than in the placebo group (2.3 ± 0.2 g/d; $P < 0.05$).

Baseline plasma cholesterol levels were comparable between the placebo group (15.6 ± 2.1 mmol/L) and the AIDM group (15.4 ± 3.2 mmol/L). While cholesterol levels did not change over time in the placebo group. AIDM rapidly (within 2 wk) and significantly decreased plasma cholesterol levels by 43% compared with the start of the study (**Fig. 2***A*). Plasma TG concentrations were significantly reduced within 2 wk by 41% in the AIDM group compared with baseline and did not change over time in the placebo group (Fig. 2*B*). The TG-lowering effect of AIDM persisted until the end of the study. Analysis of lipoprotein profiles for cholesterol demonstrated that AIDM markedly reduced cholesterol in the proatherogenic apoB-containing lipoproteins VLDL and LDL (Fig. 2*C*).

To evaluate whether AIDM exerted antiinflammatory activity during atherogenesis, plasma SAA concentrations were analyzed. At the start of the study, plasma SAA concentrations did not differ between the groups (Fig. 2*D*). They decreased 40% in the AIDM group within 2 wk and this persisted until the end of the study. Plasma SAA concentrations in the placebo group did not change.

The placebo group developed atherosclerosis (total lesion area of 59,700 ± 11,000 μm^2), whereas AIDM treatment strongly reduced atherosclerosis development ($P < 0.001$; **Fig. 3***A*,*B*). Placebo-treated mice had 6.8 ± 2.5 lesions/mouse and AIDM treatment reduced the lesion number by 92% (Fig. 3*C*; $P < 0.001$). These data indicate that AIDM strongly inhibits atherogenesis and suggest that AIDM interferes in processes critical for early lesion formation.

Monocyte adhesion is one of the early events in atherosclerotic lesion development. This process is mediated through cellular

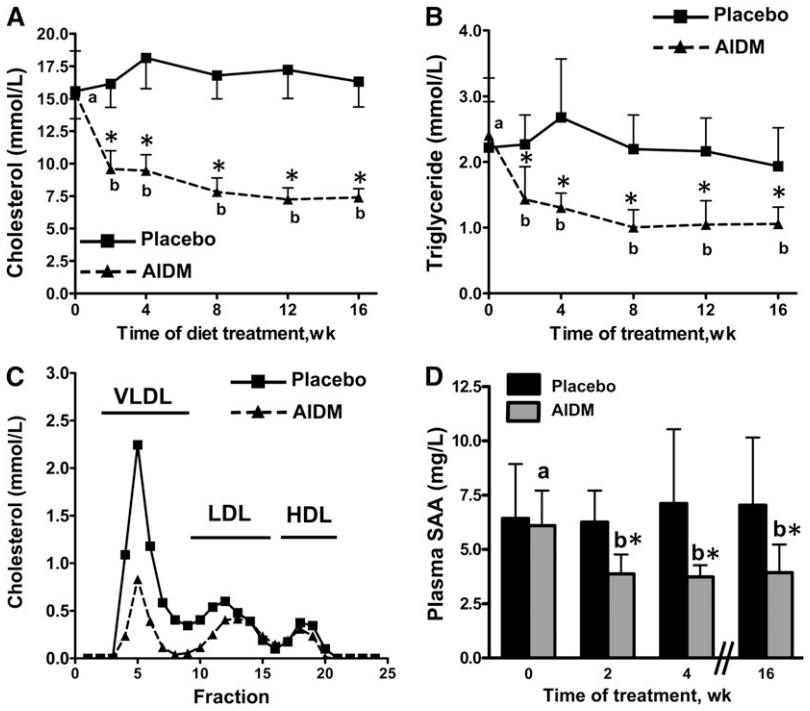

FIGURE 2 Plasma cholesterol (*A*), and TG (*B*) concentrations, lipoprotein distribution (*C*), and SAA concentrations (*D*) in female ApoE*3Leiden mice fed an atherogenic HC diet with placebo or AIDM for 16 wk. Values are mean ± SD, $n = 15$. Within a diet group, labeled means without a common letter differ, $P < 0.05$. *Different from placebo at that time, $P < 0.05$.

adhesion molecules such as ICAM-1 and E-selectin. Immunohistochemical staining of the vasculature showed that mice in the AIDM group expressed less ICAM-1 on endothelial cells compared with mice in the placebo group (**Fig. 4***A*). Subsequent quantification of ICAM-1 immunoreactivity showed that 69 ± 13% of the endothelial cells of the placebo group expressed ICAM-1 and that the percentage of ICAM-1–positive endothelial cells in the AIDM group was reduced ($P < 0.01$; Fig. 4*B*). Because the observed difference in ICAM-1 might reflect the difference in atherosclerosis, we measured plasma adhesion molecule E-selectin concentrations.

The placebo group and the AIDM group had comparable baseline plasma E-selectin concentrations (Fig. 4*C*). Compared with baseline (t0), AIDM treatment significantly reduced E-selectin levels within 2 wk, demonstrating that AIDM has a rapid anti-inflammatory effect on the vasculature. E-selectin concentrations of AIDM-treated mice remained significantly lower than those of the placebo-treated mice at the end of the study.

Discussion

The diets and eating habits in modern societies are associated with unfavorable effects on risk factors of CVD, e.g. increased circulating levels of atherogenic lipids (VLDL/LDL-cholesterol and TG) and inflammation markers such as CRP, SAA, fibrinogen, E-selectin, and ICAM-1. Because of the complex and multifactorial nature of the atherosclerotic disease process, we hypothesized that a mixture of putative beneficial dietary components would simultaneously act on multiple risk factors and thereby may constitute an effective nutrition-based strategy for preventing CVD.

We demonstrate here that a mixture containing fish oil, resveratrol, lycopene, catechin, d-α-tocopherol, and vitamin C (AIDM) reduces lipid and inflammatory risk factors of CVD and that long-term AIDM treatment strongly attenuates the development of atherosclerosis by inhibiting early processes crucial for disease initiation.

To investigate the health effects of AIDM, we used 2 transgenic mouse models, human-CRP transgenic mice and human ApoE*3Leiden transgenic mice. The background of these mice is C57BL/6, but the mice carry the human transgenes, including respective human regulatory elements. Because mouse-CRP is not an inflammation marker, human-CRP mice allow the investigation of the effects of an intervention on the expression of CRP, one of the most sensitive human inflammation markers and a

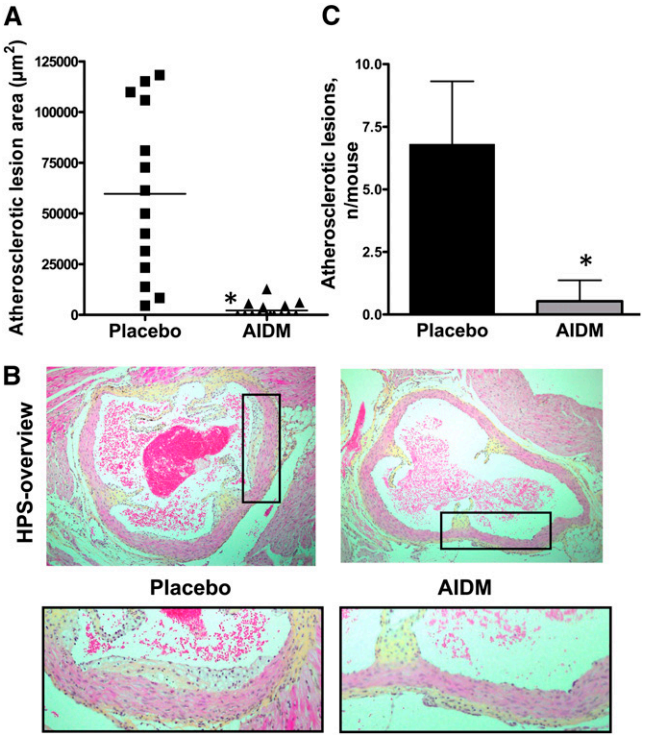

FIGURE 3 Quantitative analysis (*A*), representative pictures (*B*), and lesion number (*C*) of atherosclerosis in female ApoE*3Leiden mice fed an atherogenic HC diet with placebo or AIDM for 16 wk. Values are mean ± SD, $n = 14$ (placebo-treated) and 15 (AIDM-treated mice). *Different from placebo, $P < 0.001$.

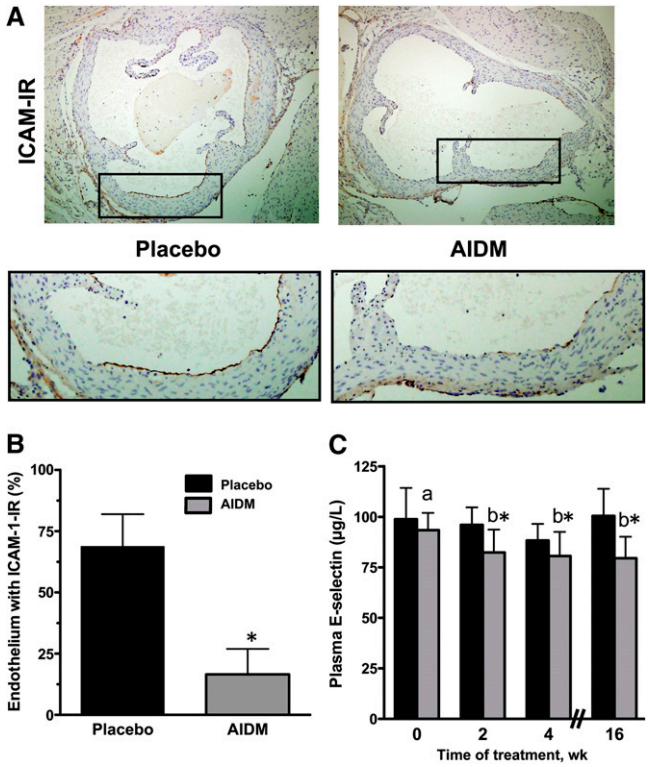

FIGURE 4 Representative pictures (*A*) and quantitative analysis of ICAM-1 (*B*) and plasma analysis of E-selectin (*C*) in female ApoE*3Leiden mice fed an atherogenic HC diet placebo or AIDM for 16 wk. Values are mean ± SD, $n = 14$ (placebo-treated mice) and 15 (AIDM-treated mice). Within a diet group, means without a common letter differ, $P < 0.05$. *Different from placebo at that time, $P < 0.05$.

well-established predictor of future cardiovascular events (23). The second model, ApoE*3Leiden mice, is an established atherosclerosis model (17). In contrast to other models of atherosclerosis (ApoE−/− and LDLR−/− mice), ApoE*3Leiden mice are sensitive to hypolipidemic actives (e.g. statins, fibrates, fish oil) and they show a cholesterol-lowering response. Unlike ApoE−/− and LDLR−/− mice, ApoE*Leiden mice are not genetically deficient for components that are necessary to metabolize lipids and to clear apoB-containing lipoproteins from the circulation. Because of these unique translational characteristics, human-CRP transgenic mice and ApoE*3Leiden mice are referred to as humanized mouse models for studying inflammation and atherosclerosis, respectively.

We found that AIDM did not alter human CRP levels in human-CRP transgenic mice fed a nonpurified diet (i.e. under a healthy dietary condition). This observation is consistent with previous findings in healthy human volunteers whose baseline CRP levels were also not affected by AIDM (14). IL-1β and IL-6 are the main inducers of CRP and are also involved in the process of atherosclerosis (24). AIDM suppressed a mild stimulation of CRP with IL-1β by ~54%. IL-1β directly activates NF-κB and CCAAT/enhancer binding protein-β (C/EBPβ) transcription factors to stimulate CRP gene expression (15) as well as the expression of IL-6, which controls STAT3-mediated transcription of CRP and another cardiovascular risk factor, fibrinogen, which is mainly regulated by STAT3 and C/EBPβ (15,25). Under the conditions applied, the IL-1β–stimulated induction of fibrinogen was fully blocked. This different efficacy of AIDM in quenching CRP and fibrinogen induction suggests that AIDM only partly quenches the activation of NF-κB but fully blocks STAT3 and/or C/EBPβ activation. A possible mechanistic explanation for the antiinflammatory effect of AIDM might be that specific fatty acids that are present in AIDM may activate PPARα, a potent and global suppressor of the IL-6–mediated acute phase response (25), which also physically inactivates NF-κB (26).

The activation of PPARα would be consistent with the reduction of plasma TG seen in both human-CRP transgenic mice and ApoE*3Leiden transgenic mice. Activation of PPARα increases β-oxidation of fatty acids, resulting in a marked TG-lowering effect. A recent study in dyslipidemic volunteers treated with a supplement of fish oils and vitamin E reported cholesterol- and TG-lowering as well as antiinflammatory effects, which is in line with the observations made herein (27).

It was documented that epigallocatechin gallate in green tea extract, lycopene from tomato extract, and α-tocopherol can reduce NF-κB activation (28–30). Because AIDM contains all of these active compounds, this may possibly explain the reduced expression of NF-κB–regulated factors (e.g. CRP, SAA, and E-selectin) in AIDM-treated mice.

In a previous study (31), we found that feeding ApoE*3Leiden transgenic mice an atherogenic diet activates specific signaling pathways (IL-1, TNFα, PDGF, IFNγ) that lead to NF-κB, C/EBPβ, and STAT3 activation and that these inflammatory pathways are not activated in ApoE*3Leiden transgenic mice fed a nonpurified diet. Consistent with this, AIMD reduced the inflammatory state under experimental conditions of atherosclerosis (atherogenic diet feeding of ApoE*3Leiden mice) and cytokine-induced inflammation (IL-1β stimulated human-CRP transgenic mice) but did not have an effect on baseline CRP levels of unstimulated mice fed a nonpurified diet.

We found that AIDM treatment markedly reduced plasma TG levels within a few weeks. Comparable TG-lowering effects were found in humans treated with AIDM (14). In humans, AIDM had no plasma LDL cholesterol-lowering effect, whereas in ApoE*3Leiden mice, plasma cholesterol confined to VLDL and LDL was strongly reduced. This apparent discrepancy may be due to a different health state; ApoE*3Leiden mice received an atherogenic diet to establish a condition of dyslipidemia (increased VLDL and LDL cholesterol), whereas human volunteers were healthy individuals with normal plasma cholesterol levels that possibly could not be further lowered with AIDM.

Analysis of atherosclerotic lesions revealed that AIDM-treated mice developed markedly fewer lesions than placebo-treated controls. This indicates that AIDM interferes with processes relevant for lesion initiation and that it protects against onset of the disease. The recruitment of inflammatory cells from the circulation and their infiltration into the vasculature is an important early stage process and is predominantly mediated by adhesion molecules such as E-selectin and ICAM-1, which are expressed on the endothelial surface. Indeed, AIDM diminished endothelial ICAM-1 expression in the aortic root and plasma E-selectin levels were significantly reduced with AIDM. In previous studies, reduced vascular inflammation and improved endothelial function were achieved with resveratrol (32), lycopene (33), green tea catechins (28), and vitamin E (34), all of which are present in AIDM. The 2 vitamins in AIDM, E and C, can affect various metabolic pathways and diminish the oxidation of circulating lipids and thereby contribute to the antiatherogenic effect observed (35,36). Because AIDM reduced vascular inflammation already at an early time point, i.e. before atherosclerosis became manifest, the effect of AIDM can be viewed as directly atheroprotective.

AIDM had a remarkably strong effect on atherosclerosis that exceeds the effect of many pharmaceuticals tested in ApoE*3 Leiden mice under comparable experimental conditions (17). The potency of AIDM may be due to the simultaneous action of its constituents (and metabolites) on multiple targets, including plasma lipids (hepatic lipid metabolism), hepatic inflammation (SAA, CRP, and fibrinogen), and vascular inflammation (E-selectin, ICAM). Such broad effects are typically not seen with single nutrients (37,38). Our findings support the concept of combination strategies with several bioactive nutrients and a systems-based, multi-target approach for complex multifactorial diseases, such as type 2 diabetes.

Our study demonstrates that a dietary mix of fish oil, resveratrol, lycopene, catechin, d-α-tocopherol, and vitamin C (AIDM) that was shown to be well tolerated in humans improves lipid and inflammatory risk factors of CVD in humanized models of disease. Most importantly, long-term treatment with AIDM strongly reduces disease endpoints, i.e. atherosclerotic lesion load and lesion number, further underlining its benefit for disease prevention.

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