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STATE-OF-THE-ART PAPER

The Effects of Diet on Inflammation

Emphasis on the Metabolic Syndrome

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Reducing the incidence of coronary heart disease with diet is possible. The main dietary strategies include adequate omega-3 fatty acids intake, reduction of saturated and trans-fats, and consumption of a diet high in fruits, vegetables, nuts, and whole grains and low in refined grains. Each of these strategies may be associated with lower generation of inflammation. This review examines the epidemiologic and clinical evidence concerning diet and inflammation. Dietary patterns high in refined starches, sugar, and saturated and trans-fatty acids, poor in natural antioxidants and fiber from fruits, vegetables, and whole grains, and poor in omega-3 fatty acids may cause an activation of the innate immune system, most likely by an excessive production of proinflammatory cytokines associated with a reduced production of anti-inflammatory cytokines. The whole diet approach seems particularly promising to reduce the inflammation associated with the metabolic syndrome. The choice of healthy sources of carbohydrate, fat, and protein, associated with regular physical activity and avoidance of smoking, is critical to fighting the war against chronic disease. Western dietary patterns warm up inflammation, while prudent dietary patterns cool it down. (J Am Coll Cardiol 2006;48:677–85) © 2006 by the American College of Cardiology Foundation

A low-fat diet ($\leq 30\%$ of total calories) is still considered by many physicians to be a healthy choice for both primary and secondary prevention of cardiovascular disease (CVD) (1). An unintended consequence of emphasizing low-fat diets may have been to promote unrestricted carbohydrate intake, which reduces high-density lipoprotein cholesterol (HDL-C) and raises triglyceride levels, exacerbating the metabolic manifestations of the insulin resistance syndrome, also known as the metabolic syndrome (2,3).

Three dietary strategies may help prevent coronary heart disease (CHD) (4): 1) increase consumption of omega-3 fatty acids from fish or plant sources; 2) substitute nonhydrogenated unsaturated fats for saturated and trans-fats; and 3) consume a diet high in fruits, vegetables, nuts, and whole grains and low in refined grains (Fig. 1). The effects of diet on CHD can be mediated through multiple biologic pathways other than serum lipids, including oxidative stress, subclinical inflammation, endothelial dysfunction, insulin sensitivity, blood pressure, and thrombotic tendency (5).

Current evidence supports a central role for inflammation in all phases of the atherosclerotic process (6). Circulating markers of inflammation, such as C-reactive protein (CRP), tumor necrosis factor (TNF)-alpha, and some interleukins (IL-6, IL-18), correlate with propensity to develop ischemic events (7–9); moreover, circulating phase reactants elicited by inflammation may not only mark increased risk for vascular events but in some cases may also contribute to their pathogenesis (10). Increasing attention has also been

paid to the direct vascular effects of plasma proteins that originate from adipose tissue, especially adiponectin, which exhibits potent anti-inflammatory and antiatherosclerotic effects (11). Low plasma adiponectin levels are an independent risk factor for future development of type 2 diabetes (12), whereas high plasma adiponectin concentrations are associated with a lower risk of myocardial infarction in men (13). Moreover, it has become increasingly clear that inflammation strictly correlates with endothelial dysfunction and insulin resistance, with the best evidence coming from patients with the metabolic syndrome (14).

We put forward the hypothesis that each dietary strategy associated with a lower CHD risk may in fact be associated with lower generation of a proinflammatory milieu, which may be one important mechanism linking healthy diets to reduced CHD risk. Criteria used for study selection to be included in the current review were English language, relevance to clinicians, study design, and venue of publication. Because controlled trials with clinical end points and assessment of inflammatory markers were lacking, we gave weight to observational studies and dietary interventional trials reporting intermediate end points.

INCREASE CONSUMPTION OF OMEGA-3 FATTY ACIDS

The benefits of omega-3 fatty acids in decreasing the risk of sudden cardiac death have been demonstrated in animal, epidemiologic, metabolic, and small clinical trials (15). These compounds have long been recognized to have anti-inflammatory activity, and their use in consolidated inflammatory diseases, such as rheumatoid arthritis and Crohn's disease, is expanding (16). Omega-3 fatty acids decrease the arachidonic acid content of cell membranes,

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Abbreviations and Acronyms

ALA	=	alpha-linolenic acid
CHD	=	coronary heart disease
CRP	=	C-reactive protein
CVD	=	cardiovascular disease
IL	=	interleukin
sICAM	=	soluble intercellular adhesion molecule
sVCAM	=	soluble vascular cell adhesion molecule
TNF	=	tumor necrosis factor

resulting in the synthesis of eicosanoids that have fewer inflammatory properties than those derived from omega-6 fatty acids. Although omega-3 fatty acids may inhibit the synthesis of proinflammatory cytokines, such as TNF-alpha, IL-1, and IL-2 (17) and decrease expression of adhesion molecules on the endothelium (18), the results of ex vivo human studies investigating the effect of omega-3 fat intake on inflammatory markers are not conclusive but may have been influenced by the specificity of cells investigated and assay procedures (19).

Observational studies. In 405 healthy men and 454 healthy women, intake of omega-3 fatty acids eicosapentaenoic acid (EPA) and docosaenoic acid (DHA) was inversely associated with plasma levels of markers of TNF-alpha activity, such as soluble TNF receptors 1 and 2; moreover, high intake of both omega-3 and omega-6 fatty acids was associated with the lowest level of inflammation (20). Therefore, the combination of omega-3 and omega-6 fatty acids may work well on CHD risk, as demonstrated by other cross-sectional data indicating that the combined intake of both fatty acids is associated with a lower risk of CHD than either type of fatty acid alone (21). Another cross-sectional study (22) of 727 women from the Nurses' Health Study I cohort demonstrated lower concentrations of many markers of inflammation and endothelial activation, including CRP, IL-6, and E-selectin, among those in the highest quintile of omega-3 fatty acids compared with those in the lowest quintile. In a randomly sampled cohort of 470 healthy middle-aged women and men from the Los Angeles Atherosclerosis Study (23), increased dietary intake of omega-3 fatty acids was associated with a blunted effect of the variant 5-lipoxygenase genotype (lacking the common allele) to increase carotid artery intima-media thickness and circulating CRP levels. In the ATTICA study, those

who consumed at least 300 g of fish per week had 33% lower CRP compared with non-fish consumers (24). Lastly, in subjects with existing CHD, granulocyte DHA content was inversely associated with CRP levels (25).

Interventional studies. Changing the proportion of omega-3 fatty acids of the diet may influence circulating markers of inflammation and endothelial activation in hypercholesterolemic subjects fed 2 diets low in saturated fat and high in polyunsaturated fats (PUFA), as compared with an average American diet (26). The alpha-linolenic acid (ALA) diet (6.5% of energy from ALA) decreased CRP, vascular cell adhesion molecule-1, and E-selectin more than the linoleic acid (LA) diet (12.6% energy from LA). In another study, dietary supplementation with ALA (15 ml linseed oil per day) for 3 months in 50 dyslipidemic subjects significantly decreased CRP (38% compared with baseline values), serum amyloid A (23%), and IL-6 (10%) levels independent of lipid changes (27). Consumption of an ALA-enriched margarine was also associated with lowering of CRP levels in hypercholesterolemic subjects (28). However, most of the studies using fish oil or pure omega-3 fatty acids supplementation have failed to show any effect on CRP levels (29-33), unless the fish oil supplement was given at a high dose (14 g/day) (34).

Although some epidemiologic studies have shown an inverse correlation between dietary intake of fish or fish oil and circulating markers of inflammation, clinical trials have not yet confirmed these effects. On the other hand, ALA appears to have anti-inflammatory potential, and future studies should focus on this.

SUBSTITUTE NONHYDROGENATED UNSATURATED FATS FOR SATURATED AND TRANS-FATS

Reducing trans-fat. Higher intake of trans-fats or, to a lesser extent, saturated fats has been shown to be associated with increased CHD risk among 80,082 women in the Nurses' Health Study cohort, whereas higher intakes of polyunsaturated (nonhydrogenated) and monounsaturated fats were associated with decreased risk (35). It is not entirely clear, however, whether the effects of this substitution are primarily due to introducing beneficial effects of unsaturated fats, moving away from the detrimental effects of saturated and trans-fats, or both. Moreover, the relation with CHD risk is explained only partially by the adverse

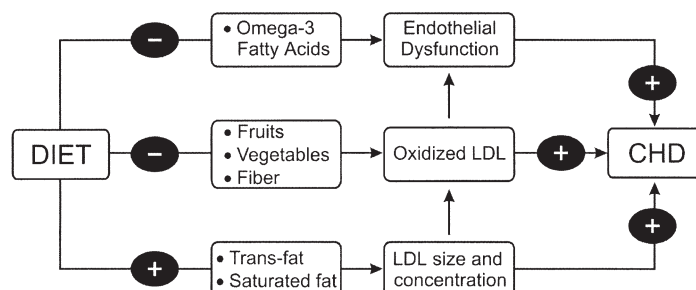


Figure 1. Some mechanisms through which unhealthy dietary patterns may lead to coronary heart disease (CHD). LDL = low-density lipoprotein.

effect of trans-fatty acids on the lipid profile (36), because the positive relation between trans-fats and cardiovascular risk is greater than one would predict based solely on its adverse effects on lipids.

OBSERVATIONAL STUDIES. In a cross-sectional study of 730 women from the Nurses' Health Study I cohort, CRP levels were 73% higher among those in the highest quintile of trans-fat intake, compared with the lowest quintile (37). Markers of endothelial activation such as E-selectin, soluble intercellular adhesion molecule (sICAM)-1, and soluble vascular cell adhesion molecule (sVCAM)-1 were also higher, suggesting that a higher intake of trans-fatty acids could favor inflammation and adversely affect endothelial function. Moreover, a previous paper from the same patient cohort showed that trans-fat intake was positively associated with IL-6 and CRP only in women with higher body mass index (38). A long-term vegetarian diet, which is low in saturated and trans-fats, is associated with better antioxidant status and CHD risk profile, and reduced concentration of CRP as compared to the diet of apparently healthy omnivores (39).

INTERVENTIONAL STUDIES. Baer et al. (40) have provided evidence that dietary fatty acids can modulate markers of inflammation in healthy humans fed controlled diets. In particular, CRP and E-selectin concentrations were higher after consumption of a trans-fatty acids diet than after consumption of a carbohydrate diet, although IL-6 concentrations were lower after consumption of an oleic acid diet than after consumption of trans- and saturated fatty acids. However, another study did not find any difference of dietary fat type on CRP levels (41).

Reducing saturated fats and cholesterol. OBSERVATIONAL STUDIES. A positive correlation between consumption of saturated fats and plasma biomarkers of inflammation has been found (42,43).

INTERVENTIONAL STUDIES. Cholesterol feeding increases CRP levels in healthy subjects but not in obese insulin-resistant patients whose CRP levels were already elevated at baseline (44). In 35 patients with primary hypercholesterolemia, an 8-week low-cholesterol/low-saturated fat diet (30% total fat, 5% saturated fat, cholesterol <200 mg/day) was associated with significant reduction of large artery stiffness (from 8.9 ± 2.0 m/s to 8.1 ± 1.9 m/s, 11%) which correlated with reduction of plasma CRP levels ($r = 0.59$) (45). One problem with this kind of intervention study is the confounding effect of weight loss that by itself may reduce inflammation and improve endothelial function (46). Moreover, energy-restricted low-fat and low-carbohydrate diets both significantly decreased several biomarkers of inflammation, such as CRP, TNF-alpha, IL-6, and ICAM-1, leading to suggest that, in the short-term, weight loss may be the driving force underlying the reductions in most of the inflammatory biomarkers (47).

CONSUME A DIET HIGH IN FRUITS, VEGETABLES, NUTS, AND WHOLE GRAINS AND LOW IN REFINED GRAINS, FRUITS, AND VEGETABLES

An inverse association between fruit and vegetable consumption and CVD has repeatedly been reported (4,48). However, only few studies have related fruit and vegetable consumption to inflammatory status.

Observational studies. Data from the Massachusetts Hispanic Elders Study (49) obtained from 445 Hispanic and 154 non-Hispanic white elders showed that greater frequency of fruit and vegetable intake was associated with lower CRP and homocysteine concentrations. With each additional serving of fruit and vegetable intake, the risk of having high CRP (>10 mg/l) and homocysteine concentrations decreased by 21% and 17%, respectively. Unfortunately, the study used a nonsensitive assay for CRP, limiting the ability to extend the association at the lower end of CRP distribution.

The antioxidant components of fruit and vegetables, including vitamins and flavonoids, are supposed to contribute to their anti-inflammatory effect (50). Observational studies have shown an inverse association between dietary total antioxidant capacity, serum carotenoids and vitamins, and markers of inflammation (51,52). The intake of dietary fiber may play a role as well. Using data from the National Health and Nutrition Examination Survey 1999 to 2000, Ajani et al. (53) examined the relation between dietary fiber and CRP in 3,920 participants. Dietary fiber intake was inversely associated with serum CRP concentrations: The adjusted odds ratio for increased CRP levels (>3 mg/l) was 0.59 ($p = 0.006$) for the highest quintile of fiber intake compared with the lowest. The results were not affected after exclusion of persons with diabetes, cancer, CVD, or CRP levels >10 mg/l. The results of Ajani et al. (53) confirmed the previous findings of King et al. (43).

Interventional studies. Drinking 500 ml/day of high-pressurized orange juice for 14 days reduced plasma CRP by 40% and 56% in men and women, respectively (54). A randomized controlled 4-week trial in nonsmoking men showed that subjects assigned to high consumption (8 servings/day) of carotenoid-rich vegetables and fruit had significantly reduced CRP levels compared with those who consumed 2 servings/day (55). In contrast, another randomized controlled parallel 6-week dietary intervention trial in healthy volunteers failed to show any effect of diets differing markedly in the amounts of vegetables, berries, and apple on CRP levels (56). On the other hand, the addition of antioxidant supplements (57) or vegetables to the diet (58) has been shown to reverse the increase in circulating adhesion molecules (sICAM-1 and sVCAM-1), proinflammatory cytokines (IL-6 and TNF-alpha), and endothelial dysfunction induced by a single high-fat (saturated) meal consumption. More work is needed to characterize fruits and vegetables with anti-inflammatory effects and the substances responsible.

NUTS

An inverse association between nut consumption and CHD risk has been consistently found (4). Nuts are rich in monounsaturated and polyunsaturated fatty acids, which makes them a palatable choice of healthy fats. Monounsaturated fats may contribute to decreased CHD risk by amelioration of lipid profile (59), by reducing postprandial triglyceride concentrations (60), and by decreasing soluble inflammatory adhesion molecules in patients with hypercholesterolemia (61). Moreover, the relatively high arginine content of nuts has been suggested as one of the potential biologic mechanisms for their cardioprotective effect, because consumption of arginine-rich foods is associated with lower CRP levels (62).

REDUCE REFINED GRAINS

Carbohydrate type affects CHD risk. Refined carbohydrates are highly processed, resulting in removal of fiber, vitamins, minerals, phytonutrients, and essential fatty acids. High intake of refined starches and sugars causes rapid swings in blood glucose and insulin levels, may increase hunger, and may elevate free fatty acid levels (63). Acute hyperglycemia in normal humans impairs endothelium-dependent vasodilation (64) and reduces nitric oxide availability (65). By its mass action, postprandial hyperglycemia leads to increased tissue glucose uptake and metabolism by ordinarily minor pathways (Fig. 2). One major consequence of this activation is unrestrained production of free radicals which may promote atherogenesis by different mechanisms (66,67). Strictly related to this is the emerging evidence that CHD may be more strongly related to post-load glucose hyperglycemia than to fasting hyperglycemia (68). Although the mechanisms underlying these associations are not fully clear, recent data indicate that short-term acute hyperglycemia may increase circulating levels of free radicals and proinflammatory cytokines, such as IL-6, IL-18, and TNF-alpha (69), providing a plausible explanation for the deleterious effects of rapid glycaemic waves on vasculature.

Observational studies. Festa et al. (70) evaluated the relationship of CRP with fasting and post-challenge plasma glucose in nondiabetic subjects from the Insulin Resistance Atherosclerotic Study: after adjustment for many demographic and anthropometric variables, a significant association was found for 2 h glucose only with CRP levels. Moreover, a cross-sectional analysis conducted in 780 diabetic men from the Health Professionals' Follow-Up Study (71) has recently shown that diets low in glycaemic load and high in fiber may increase plasma adiponectin concentrations in diabetic patients.

Interventional studies. Meal modulation of circulating inflammatory and anti-inflammatory cytokines may play a role in the detrimental or beneficial effects of different types of carbohydrates. For example, the fiber content of a high-carbohydrate meal may influence plasma levels of adiponectin and IL-18: the higher the quantity of fiber in

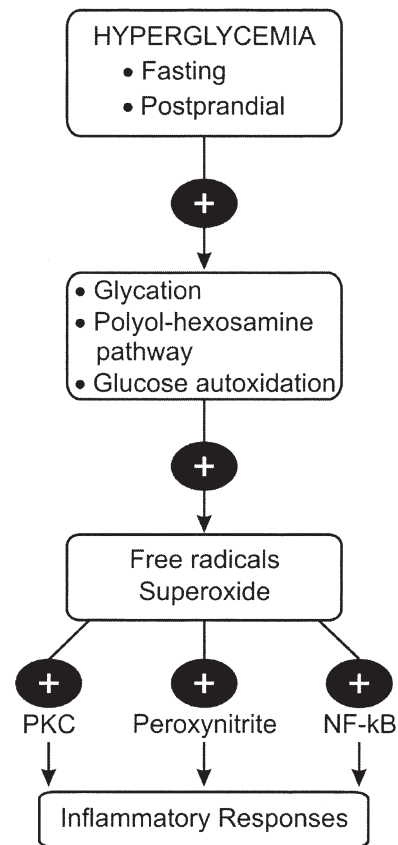


Figure 2. Mechanisms through which hyperglycemia may generate inflammatory responses. NF-kB = nuclear factor kappa B; PKC = protein kinase C.

the load, the greater the inhibition of plasma IL-18 and stimulation of adiponectin (72). On the other hand, in overweight subjects a high consumption of sugar-sweetened foods and drinks increased haptoglobin and transferrin but had, at best, only a limited influence on CRP (73).

ALCOHOL

Alcohol, mostly wine, represents an important component of the Mediterranean diet; moreover, moderate alcohol intake has consistently been shown to be associated with a lower risk for fatal and nonfatal cardiovascular disease (74).

Observational studies. Lower concentrations of several markers of inflammation, including CRP, have been reported among moderate consumers of alcohol in individuals with and without pre-existing CVD (75-77). The association has recently been confirmed in large prospective studies, in both Europe (78) and the U.S. (79), suggesting that ethanol itself might be largely responsible for the potential anti-inflammatory effects of beer, wine, or liquor.

Interventional studies. Sierksma et al. (80) demonstrated a significant reduction of CRP concentrations and fibrinogen after 3 weeks of diet-controlled consumption of 4 (men) or 3 (women) glasses of beer. Moreover, a 4-week consumption of 30 g/day of red wine led to a significant decrease in CRP (21%) in healthy adult men (81).

THE WHOLE DIET APPROACH

The role of overall dietary patterns in predicting long-term risk of CHD has recently been demonstrated (82). In practical terms, a prudent dietary pattern is characterized by the choice of foods that satisfy all the strategies so far discussed for reducing CHD risk, that is, a higher intake of fruits, vegetables, legumes, whole grains, poultry, and fish. Needless to say, this pattern is associated with lower risk of CHD, as opposed to a Western dietary pattern (higher intakes of red and processed meat, sweets and desserts, potatoes and French fries, and refined grains) which is associated with an increased risk. As modern eating patterns of Western societies generate an almost endless postprandial phase through the day, a chronic activation of the innate immune system could exist during most parts of the day.

Observational studies. In a cross-sectional study of 732 women from the Nurses' Health Study I cohort (83), a prudent pattern was inversely associated with plasma concentrations of CRP and E-selectin, whereas the Western pattern showed a positive relation with CRP, E-selectin, ICAM-1, and sVCAM-1 after adjustment for all confounders. A positive correlation between the Western pattern and higher plasma CRP levels has also been found in men (42). Moreover, a Western-type dietary pattern that increases chronic inflammation raises the risk of developing type 2 diabetes (84). In contrast, another observational study found that CRP levels were only marginally associated with dietary and lifestyle factors in 760 subjects of both genders (85).

Interventional studies. Jenkins et al. (86) found a reduction of CRP levels (28% vs. baseline) in hyperlipidemic patients following a whole diet approach, which was comparable to statin therapy (33% reduction of CRP levels) and independent of changes in body weight. The diet, which was low in saturated fat and included viscous fibers, almonds, soy protein, and plant sterols, also induced reduction in lipids that was comparable to lovastatin therapy.

Although the whole diet approach cannot allow determining whether the benefit is due to an added nutrient, a removed nutrient, or a combination of both, the evidence suggests that numerous dietary changes contribute to the reduction in chronic disease risk.

THE MEDITERRANEAN DIET

The Mediterranean-style diet can be considered a good example of a prudent dietary pattern.

Observational studies. In a large prospective survey involving about 22,000 adults from Greece (the Greek cohort of the EPIC [European Prospective Investigation into Cancer and Nutrition] study), an inverse correlation between a greater adherence to a Mediterranean-style diet and death has been shown (87). In particular, approximately a 2/9 increment in the Mediterranean diet score was associated with a 25% reduction in total mortality and a 33% reduction in CHD mortality. Chrysohoou et al. (88) eval-

uated 1,514 men and 1,528 women from the ATTICA study in Greece and observed that greater adherence to a Mediterranean-style diet was associated with 20% lower CRP and 17% lower IL-6 compared with those in the lowest tertile of adherence, after various adjustments were made. In the Nurses' Health Study, a Mediterranean diet index score was associated with lower concentrations of biomarkers of inflammation and endothelial dysfunction (89).

Interventional studies. The only 2 randomized trials that tested the whole-diet approach in secondary prevention of CHD with hard clinical end points were conducted using Mediterranean-style diets. In the Lyon Diet Heart Study (90), the most frequent nonfatal events were new acute myocardial infarction and episodes of unstable angina that are commonly due to rupture of an atherosclerotic plaque. The risk of these 2 end points was reduced by about 70% by the Mediterranean diet, leading to the hypothesis that biologic changes associated with it resulted in a significant local anti-inflammatory effect. Singh et al. (91) tested an "Indo-Mediterranean diet" in 1,000 patients in India with existing coronary disease or at high risk for coronary disease. Compared with the control diet, the intervention diet—characterized by increased intake of mustard or soybean oil, nuts, vegetables, fruits, and whole grains—reduced the rate of fatal myocardial infarction by one-third and the rate of sudden death from cardiac causes by two-thirds. Because an "expression of concern" (92) has recently been issued about this last paper, the Lyon Heart Study remains the only randomized trial that tested the whole diet approach with a Mediterranean-style diet.

It must be pointed out that much confusion still exists about the meaning of the Mediterranean diet: a recent review (1) included the DART (Diet And Reinfarction Trial) study (93) and the GISSI (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico acuto)-Prevenzione study (94) among the controlled trials using a Mediterranean-style diet approach. Obviously, the DART study tested the effect of fish intake, whereas omega-3 supplements were used in the GISSI-Prevenzione.

THE METABOLIC SYNDROME: A NEW TARGET FOR THE WHOLE DIET APPROACH

The metabolic syndrome represents a cluster of several risk factors for atherosclerosis, including visceral obesity, atherogenic dyslipidemia, hyperglycemia, and hypertension, and is strictly associated with subclinical inflammation (14,95). Because the metabolic syndrome is highly prevalent in the U.S. (96) and is a risk factor for CHD (14,95), interventions aimed at reducing its prevalence hopefully will contribute to decreasing the burden of accompanying disease.

Observational studies. Williams et al. (97) showed that dietary patterns close to the Mediterranean diet, rich in fruit and vegetables and high in monounsaturated fats, were negatively associated with features of the metabolic syn-

drome. More recently, a reduced prevalence of the metabolic syndrome (38% lower) was observed among subjects of the Framingham Offspring Study consuming the highest intake of cereal fiber, compared those with the lowest intake (98). In the ATTICA study (99), adherence to a Mediterranean-style dietary pattern was associated with a 20% lower risk of having the metabolic syndrome.

Interventional studies. Esposito et al. (100) explored possible mechanisms underlying a dietary intervention and randomized 180 patients (99 men, 81 women) with the metabolic syndrome to a Mediterranean-style diet (instructions about increasing daily consumption of whole grains, vegetables, fruits, nuts, and olive oil) versus a cardiac-prudent diet with fat intake less than 30%. After 2 years, body weight decreased more in the intervention group than in the control group, but even after controlling for weight loss, inflammatory markers, such as IL-6, IL-7, IL-18, and CRP, and insulin resistance declined more in the intervention than in the control group, and endothelial function improved. Only 40 patients in the intervention group still had metabolic syndrome after 2 years compared with 78 patients on the control diet; thus, the prevalence of the metabolic syndrome was reduced approximately by one-half (Fig. 3). Because a similar decrease in the prevalence of the metabolic syndrome has been obtained with rimonabant, a cannabinoid receptor blocker, in a group of obese patients after 1 year of treatment, with a 30% rate of discontinuation for side effects (101,102), the whole diet approach seems particularly intriguing and promising to reduce the cardiovascular burden associated with the metabolic syndrome.

It should be pointed out that a recent controlled trial investigating the effect of a Mediterranean diet in 101 patients with established and treated CHD failed to show



Figure 3. Effects of a 2-year dietetic approach with a Mediterranean-style diet in patients with the metabolic syndrome. The number of components of the syndrome was reduced approximately by one-half in the intervention group (100).

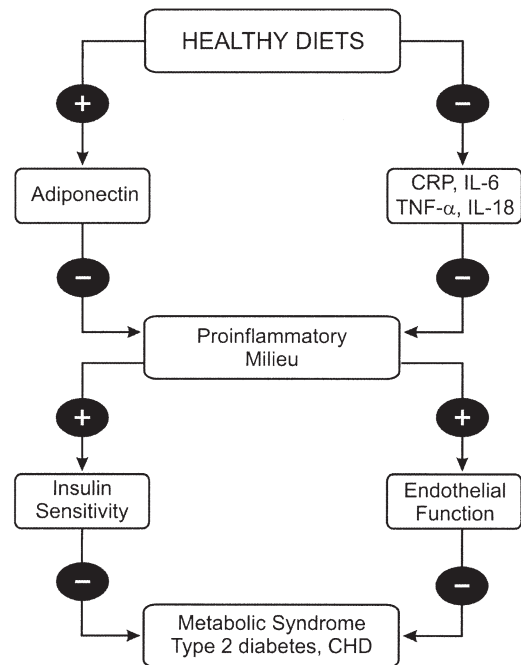


Figure 4. Healthy dietary patterns contribute to a lower generation of a proinflammatory milieu, which in turn may decrease the incidence of metabolic syndrome, type 2 diabetes, and coronary heart disease (CHD). CRP = C-reactive protein; IL = interleukin; TNF = tumor necrosis factor.

any effect on markers of inflammation, including high-sensitivity CRP, after 1 year (103).

CONCLUSIONS

Dietary patterns high in refined starches, sugar, and saturated and trans-fatty acids and poor in natural antioxidants and fiber from fruits, vegetables, and whole grains may cause an activation of the innate immune system, most likely by an excessive production of proinflammatory cytokines associated with a reduced production of anti-inflammatory cytokines. This imbalance may favor the generation of a proinflammatory milieu, which in turn produces endothelial dysfunction at the vascular level, and ultimately predisposes susceptible people to increased incidence of the metabolic syndrome and CHD. All the dietary strategies hitherto demonstrated to reduce CHD risk are associated with reduced inflammation, which may explain, at least in part, their benefit at the vascular level (Fig. 4). An inflammatory state may also prevent a favorable responsiveness of serum lipids to a reduced-fat low-cholesterol diet (104); if confirmed, these findings might generate the gloomy perspective in which the inflammatory state triggered by “Western” dietary patterns may in turn impair the lipid-lowering effect of low-fat diets.

Low consumption of fruit and vegetables, together with physical inactivity, are now among the top 10 causes of mortality in developed countries (105). Because cardiovascular diseases, various forms of cancer, and diabetes combine to make up nearly 70% of all deaths in the U.S. (106), and

the mounting epidemic of obesity (107,108) and the metabolic syndrome (88) is likely to increase the prevalence of most of these diseases in the near future, adoption of a healthy lifestyle is paramount to reducing chronic disease risk. Therefore, the choice of healthy sources of carbohydrates, fat, and proteins, associated with regular physical activity and avoidance of smoking, is critical to fighting the war against chronic disease. This seems particularly important for individuals who carry additional risk factors, such as type 2 diabetes mellitus, obesity, and the metabolic syndrome, and fail, as most do, to have a consistent and long-term weight loss.

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REFERENCES

1. Parikh P, McDaniel MC, Ashen MD, et al. Diets and cardiovascular disease. An evidence-based assessment. *J Am Coll Cardiol* 2005;45:1379-87.
2. Grundy SM. Hypertriglyceridemia, insulin resistance, and the metabolic syndrome. *Am J Cardiol* 1999;83:25F-9F.
3. Weinber SL. The diet-heart hypothesis: a critique. *J Am Coll Cardiol* 2004;43:731-3.
4. Hu FB, Willett WC. Optimal diets for prevention of coronary heart disease. *JAMA* 2002;288:2569-78.
5. Hu FB, Willett WC. Diet, nutrition, and coronary heart disease. In: Douglas PS, editor. *Cardiovascular Health and Disease in Women*. 2nd edition. Philadelphia, PA: WB Saunders, 2002:71-92.
6. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation* 2002;105:1135-43.
7. Ridker PM, Rifai N, Stampfer MJ, et al. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation* 2000;101:1767-72.
8. Ridker PM, Rifai N, Pfeffer M, et al. Elevation of tumor necrosis factor- α and increased risk of recurrent coronary events after myocardial infarction. *Circulation* 2000;101:2149-53.
9. Blankenberg S, Tiret L, Bickel C, et al. Interleukin-18 is a strong predictor of cardiovascular death in stable and unstable angina. *Circulation* 2002;106:24-30.
10. Lau DCW, Dhillon B, Yan H, et al. Adipokines: molecular links between obesity and atherosclerosis. *Am J Physiol Heart Circ Physiol* 2005;288:H2031-41.
11. Tataranni PA, Ortega E. A burning question. Does an adipokine-induced activation of the immune system mediate the effect of overnutrition on type 2 diabetes? *Diabetes* 2005;54:917-27.
12. Lindsay RS, Funahashi T, Hanson RL, et al. Adiponectin and development of type 2 diabetes in the Pima Indian population. *Lancet* 2002;360:57-8.
13. Pischon T, Girman CJ, Hotamisligil GS, et al. Plasma adiponectin levels and risk of myocardial infarction in men. *JAMA* 2004;291:1730-7.
14. Esposito K, Giugliano D. The metabolic syndrome and inflammation: association or causation? *Nutr Metab Cardiovasc Dis* 2004;14:228-32.
15. Kang JX, Leaf A. Prevention of fatal arrhythmias by polyunsaturated fatty acids. *Am J Clin Nutr* 2000;71:202S-7S.
16. Connor WE. Importance of n-3 fatty acids in health and disease. *Am J Clin Nutr* 2000;71:171-5S.
17. von Shacky C. N-3 fatty acids and prevention of coronary atherosclerosis. *Am J Clin Nutr* 2000;71:224S-7S.
18. Brown AA, Hu FB. Dietary modulation of endothelial function: implications for cardiovascular disease. *Am J Clin Nutr* 2001;73:673-86.
19. Calder PC. Dietary modification of inflammation with lipids. *Proc Nutr Soc* 2002;61:345-58.
20. Pischon T, Hankinson SE, Hotamisligil GS, et al. Habitual dietary intake of n-3 and n-6 fatty acids in relation to inflammatory markers among U.S. men and women. *Circulation* 2003;108:155-60.
21. Djousse L, Pankov JS, Eckfeldt JH, et al. Relation between linolenic acid and coronary artery disease in the National Heart, Lung, and Blood Institute Family Heart Study. *Am J Clin Nutr* 2001;74:612-9.
22. Lopez-Garcia E, Schulze MB, Manson JE, et al. Consumption of (n-3) fatty acids is related to plasma biomarkers of inflammation and endothelial activation in women. *J Nutr* 2004;134:1806-11.
23. Dwyer JH, Allayee H, Dwyer KM, et al. Arachidonate 5-lipoxygenase promoter genotype, dietary arachidonic acid, and atherosclerosis. *N Engl J Med* 2004;350:4-7.
24. Zampelas A, Panagiotakis DB, Pitsavos C, et al. Fish consumption among healthy adults is associated with decreased levels of inflammatory markers related to cardiovascular disease: the ATTICA study. *J Am Coll Cardiol* 2005;46:120-4.
25. Madsen T, Skou HA, Hansen VE, et al. C-reactive protein, dietary n-3 fatty acids, and the extent of coronary artery disease. *Am J Cardiol* 2001;88:1139-42.
26. Zhao G, Etherton TD, Martin KR, et al. Dietary alpha-linolenic acid reduces inflammatory and lipid cardiovascular risk factors in hypercholesterolemic men and women. *J Nutr* 2004;134:2991-7.
27. Rallidis LS, Paschos G, Liakos GK, et al. Dietary alpha-linolenic acid decreases C-reactive protein, serum amyloid A and interleukin 6 in dyslipidemic patients. *Atherosclerosis* 2003;167:237-42.
28. Bemelmans WJ, Lefrandt JD, Feskens EJ, et al. Increased alpha-linolenic acid intake lowers C-reactive protein, but has no effect on markers of atherosclerosis. *Eur J Clin Nutr* 2004;58:1083-9.
29. Chan DC, Watts GF, Barrett PH, et al. Effect of atorvastatin and fish oil on plasma high-sensitivity C-reactive protein concentrations in individuals with visceral obesity. *Clin Chem* 2002;48:877-83.
30. Geelen A, Brouwer IA, Schouten EG, et al. Intake of n-3 fatty acids from fish does not lower serum concentrations of C-reactive protein in healthy subjects. *Eur J Clin Nutr* 2004;58:1440-2.
31. Madsen T, Christensen JH, Blom M, Schmidt EB. The effect of dietary n-3 fatty acids on serum concentrations of C-reactive protein: a dose-response study. *Br J Nutr* 2003;89:517-22.
32. Vega-Lopez S, Kaul N, Devaraj S, et al. Supplementation with omega3 polyunsaturated fatty acids and all-rac alpha-tocopherol alone or in combination failed to exert an antiinflammatory effect in human volunteers. *Metabolism* 2004;53:236-40.
33. Jellema A, Plat J, Mensink RP. Weight reduction, but not a moderate intake of fish oil, lowers concentrations of inflammatory markers and PAI-1 antigen in obese men during the fasting and postprandial state. *Eur J Clin Invest* 2004;34:766-73.
34. Ciubotaru I, Lee YS, Wander RC. Dietary fish oil decreases C-reactive protein, interleukin-6, and triacylglycerol to HDL-cholesterol ratio in postmenopausal women on HRT. *J Nutr Biochem* 2003;14:513-21.
35. Hu FB, Stampfer MJ, Manson JE, et al. Dietary fat intake and the risk of coronary heart disease in women. *N Engl J Med* 1997;337:1491-9.
36. Judd JT, Baer DJ, Clevidence BA, et al. Dietary cis and trans monounsaturated and saturated FA and plasma lipids and lipoproteins in men. *Lipids* 2002;37:123-31.
37. Lopez-Garcia E, Schulze MB, Meigs JB, et al. Consumption of trans fatty acids is related to plasma biomarkers of inflammation and endothelial dysfunction. *J Nutr* 2005;135:562-6.
38. Mozaffarian D, Pischon T, Hankinson SE, et al. Dietary intake of trans fatty acids and systemic inflammation in women. *Am J Clin Nutr* 2004;79:606-12.
39. Szeto YT, Kwok TC, Benzie IF. Effects of long-term vegetarian diet on biomarkers of antioxidant status and cardiovascular disease risk. *Nutrition* 2004;20:863-6.
40. Baer DJ, Judd JT, Clevidence BA, Tracy RP. Dietary fatty acids affect plasma markers of inflammation in healthy men fed controlled diets: a randomized cross-over study. *Am J Clin Nutr* 2004;79:969-73.
41. Lichtenstein AH, Erkkila AT, Lamarche B, et al. Influence of hydrogenated fat and butter on CVD risk factors: remnant-like

- particles, glucose and insulin, blood pressure and C-reactive protein. *Atherosclerosis* 2003;171:97-107.
42. Fung TT, Rimm EB, Spiegelman D, et al. Association between dietary patterns and plasma biomarkers of obesity and cardiovascular disease risk. *Am J Clin Nutr* 2001;73:61-7.
 43. King DE, Egan BM, Geesey ME. Relation of dietary fat and fiber to elevation of C-reactive protein. *Am J Cardiol* 2003;92:1335-9.
 44. Tannock LR, O'Brien KD, Knopp RH, et al. Cholesterol feeding increases C-reactive protein and serum amyloid A levels in lean insulin-sensitive subjects. *Circulation* 2005;111:3058-62.
 45. Pirro M, Schillaci G, Savarese G, et al. Attenuation of inflammation with short-term dietary intervention is associated with a reduction of arterial stiffness in subjects with hypercholesterolaemia. *Eur J Cardiovasc Prev Rehabil* 2004;11:497-502.
 46. Ziccardi P, Nappo F, Giugliano G, et al. Reduction of inflammatory cytokine concentrations and improvement of endothelial functions in obese women after weight loss over one year. *Circulation* 2002;105:804-9.
 47. Sharman MJ, Volek JS. Weight loss leads to reductions in inflammatory biomarkers after a very-low carbohydrate diet and a low-fat diet in overweight men. *Clin Sci (Lond)* 2004;107:365-9.
 48. Rissanen TH, Voutilainen S, Virtanen JK, et al. Low intake of fruits, berries, and vegetables is associated with excess mortality in men: the Kuopio Ischaemic Heart Disease Risk Factors (KIHD) Study. *J Nutr* 2003;133:199-204.
 49. Gao X, Bermudez OI, Tucker KL. Plasma C-reactive protein and homocysteine concentrations are related to frequent fruit and vegetable intake in Hispanic and non-Hispanic white elders. *J Nutr* 2004;134:913-8.
 50. Maron DJ. Flavonoids for reduction of atherosclerotic risk. *Curr Atheroscler Rep* 2004;6:73-8.
 51. Brighenti F, Valtuena S, Pellegrini N, et al. Total antioxidant capacity of the diet is inversely and independently related to plasma concentration of high-sensitivity C-reactive protein in adult Italian subjects. *Br J Nutr* 2005;93:619-25.
 52. van Herpen-Broekmans VM, Klopping-Ketelaars IA, Bots ML, et al. Serum carotenoids and vitamins in relation to markers of endothelial function and inflammation. *Eur J Epidemiol* 2004;19:915-21.
 53. Ajani UA, Ford ES, Mokdad AL. Dietary fiber and C-reactive protein: findings from National Health and Nutrition Examination Survey data. *J Nutr* 2004;134:1181-5.
 54. Sanchez-Moreno C, Cano MP, de Ancos B, et al. High-pressurized orange juice consumption affects plasma vitamin C, antioxidant status and inflammatory markers in healthy humans. *J Nutr* 2003;133:2204-9.
 55. Watzl B, Kulling SE, Moseneder J, Barth SW, Bub A. A 4-wk intervention with high intake of carotenoid-rich vegetables and fruit reduces plasma C-reactive protein in healthy, nonsmoking men. *Am J Clin Nutr* 2005;82:1052-8.
 56. Freese R, Vaarala O, Turpeinen AM, Mutanen M. No difference in platelet activation or inflammatory markers after diets rich or poor in vegetables, berries and apple in healthy subjects. *Eur J Nutr* 2004;43:175-82.
 57. Nappo F, Esposito K, Cioffi M, et al. Postprandial endothelial activation in healthy subjects and in type 2 diabetic patients: role of fat and carbohydrate meals. *J Am Coll Cardiol* 2002;39:1145-50.
 58. Esposito K, Nappo F, Giugliano F, et al. Effect of dietary antioxidants on postprandial endothelial dysfunction induced by a high-fat meal in healthy subjects. *Am J Clin Nutr* 2003;77:139-43.
 59. Kris-Etherton P. Monounsaturated fatty acids and risk of cardiovascular disease. *Circulation* 1999;100:1253-8.
 60. Thomsen C, Storm H, Holst JJ, Hermansen H. Differential effects of saturated and monounsaturated fats on postprandial lipemia and glucagon-like peptide-1 responses in patients with type 2 diabetes. *Am J Clin Nutr* 2003;77:605-11.
 61. Fuentes F, Lopez-Miranda J, Sanchez E, et al. Mediterranean diet and low-fat diets improve endothelial function in hypercholesterolemic men. *Ann Intern Med* 2001;134:1115-19.
 62. Wells BJ, Mainous AG 3rd, Everett CJ. Association between dietary arginine and C-reactive protein. *Nutrition* 2005;21:125-30.
 63. Foster-Powell K, Holt SHA, Brand-Miller JC. International table of glycemic index and glycemic load values: 2002. *Am J Clin Nutr* 2002;76:5-56.
 64. Williams S, Goldfine A, Timimi F, et al. Acute hyperglycemia attenuates endothelium-dependent vasodilation in humans in vivo. *Circulation* 1998;97:1695-701.
 65. Giugliano D, Marfella R, Coppola L, et al. Vascular effects of acute hyperglycemia in humans are reversed by L-arginine. Evidence for reduced availability of nitric oxide during hyperglycemia. *Circulation* 1997;95:1783-90.
 66. Giugliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. *Diabetes Care* 1996;19:257-67.
 67. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature* 2001;414:813-20.
 68. DECODE Study Group. Glucose tolerance and mortality. Comparison of WHO and American Diabetes Association diagnostic criteria. *Lancet* 1999;354:617-21.
 69. Esposito K, Nappo F, Marfella R, et al. Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress. *Circulation* 2002;106:2067-72.
 70. Festa A, D'Agostino R Jr., Tracy RP, Haffner SM. C-reactive protein is more strongly related to post-glucose load glucose than to fasting glucose in nondiabetic subjects; the Insulin Resistance Atherosclerotic Study. *Diabetic Med* 2002;19:939-43.
 71. Qi L, Rimm E, Liu S, Rifai N, Hu FB. Dietary glycemic index, glycemic load, cereal fiber, and plasma adiponectin concentration in diabetic men. *Diabetes Care* 2005;28:1022-8.
 72. Esposito K, Nappo F, Giugliano F, et al. Meal modulation of circulating interleukin 18 and adiponectin concentrations in healthy subjects and in patients with type 2 diabetes mellitus. *Am J Clin Nutr* 2003;78:1135-40.
 73. Sorensen LB, Raben A, Astrup A. Effect of sucrose on inflammatory markers in overweight humans. *Am J Clin Nutr* 2005;82:421-7.
 74. Corrao G, Rubbiati L, Bagnardi V, et al. Alcohol and coronary heart disease: a meta-analysis. *Addiction* 2000;95:1505-23.
 75. Imhof A, Froehlich M, Brenner H, et al. Effect of alcohol consumption on systemic markers of inflammation. *Lancet* 2001;357:763-7.
 76. Albert MA, Glynn RJ, Ridker PM, et al. Alcohol consumption and plasma concentration of C-reactive protein. *Circulation* 2003;107:443-7.
 77. Stewart SH, Mainous AG, Gilbert G, et al. Relation between alcohol consumption and C-reactive protein levels in the adult US population. *J Am Board Fam Pract* 2002;15:437-42.
 78. Imhof A, Woodward M, Doering A, et al. Overall alcohol intake, beer, wine, and systemic markers of inflammation in Western Europe: results from three MONICA samples (Augsburg, Glasgow, Lille). *Eur Heart J* 2004;25:2092-100.
 79. Levitan EB, Ridker PM, Manson JE, et al. Association between consumption of beer, wine, and liquor and plasma concentration of high-sensitivity C-reactive protein in women aged 39 to 89 years. *Am J Cardiol* 2005;96:83-8.
 80. Sierksma A, Van Der Gaag MS, Klufft C, et al. Moderate alcohol consumption reduces plasma C-reactive protein and fibrinogen levels; a randomized, diet-controlled intervention study. *Eur J Clin Nutr* 2002;56:1130-6.
 81. Estruch R, Sacanella E, Badia E, et al. Different effects of red wine and gin consumption on inflammatory biomarkers of atherosclerosis: a prospective randomized crossover trial. Effect of wine on inflammatory markers. *Atherosclerosis* 2004;175:117-23.
 82. Hu FB. Dietary patterns analysis: a new direction in nutritional epidemiology. *Curr Opin Lipidol* 2002;13:3-9.
 83. Lopez-Garcia E, Schulze MB, Fung TT, et al. Major dietary patterns are related to plasma concentrations of markers of inflammation and endothelial dysfunction. *Am J Clin Nutr* 2004;80:1029-35.
 84. Schulze MB, Hoffman K, Manson JE, et al. Dietary pattern, inflammation, and incidence of type 2 diabetes in women. *Am J Clin Nutr* 2005;82:675-84.
 85. Fredrikson GN, Hedblad B, Nilsson JA, Alm R, Berglund G, Nilsson J. Association between diet, lifestyle, metabolic cardiovascular risk factors, and plasma C-reactive protein levels. *Metabolism* 2004;53:1436-42.
 86. Jenkins DJA, Kendall CWC, Marchie A, et al. Effects of a dietary portfolio of cholesterol-lowering foods vs lovastatin on serum lipids and C-reactive protein. *JAMA* 2003;290:502-10.
 87. Trichopoulos A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. *N Engl J Med* 2003;348:2599-608.

88. Chrysohoou C, Panagiotakis DB, Pitsavos C, et al. Adherence to the Mediterranean diet attenuates inflammation and coagulation process in healthy adults: the ATTICA study. *J Am Coll Cardiol* 2004;44:152–8.
89. Fung TT, McCullough ML, Newby PK, et al. Diet-quality scores and plasma concentrations of markers of inflammation and endothelial dysfunction. *Am J Clin Nutr* 2005;82:163–73.
90. de Lorgeril M, Salen P, Martin JL, et al. Mediterranean diet, traditional risk factors and the rate of cardiovascular complications after myocardial infarction. Final report of the Lyon Diet Heart Study. *Circulation* 1999;99:779–85.
91. Singh RB, Dubnov G, Niaz MA, et al. Effect of Indo-Mediterranean diet on progression of coronary disease in high risk patients: a randomized single blind trial. *Lancet* 2002;360:1455–61.
92. Horton R. Expression of concern: Indo-Mediterranean Diet Heart Study. *Lancet* 2005;366:354–6.
93. Burr ML, Gilbert JH, Holliday RM, et al. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: Diet and Reinfarction Trial (DART). *Lancet* 1989;2:757–61.
94. GISSI-Prevenzione Investigators. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. *Lancet* 1999;354:447–55.
95. Grundy SM, Brewer HB, Cleeman JI, et al. Definition of metabolic syndrome. *Circulation* 2004;109:433–8.
96. Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among U.S. adults: findings from the Third National Health and Nutrition Examination Survey. *JAMA* 2002;287:356–9.
97. Williams DE, Prevost AT, Whiclow MJ, et al. A cross-sectional study of dietary patterns with glucose intolerance and other features of the metabolic syndrome. *Br J Nutr* 2000;83:257–66.
98. McKeown NM, Meigs JB, Liu S, et al. Carbohydrate nutrition, insulin resistance, and the prevalence of the metabolic syndrome in the Framingham Offspring Cohort. *Diabetes Care* 2004;27:538–46.
99. Panagiotakis DB, Pitsavos CH, Chrysohoou C, et al. The impact of lifestyle habits on the prevalence of the metabolic syndrome among Greek adults from the ATTICA study. *Am Heart J* 2004;147:106–12.
100. Esposito K, Marfella R, Ciotola M, et al. Effect of a Mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. *JAMA* 2004;292:1440–6.
101. Van Gaal LF, Rissanen AM, Scheen AJ, et al., RIO-Europe Study Group. Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. *Lancet* 2005;365:1389–97.
102. Esposito K, Giugliano D. Effect of rimonabant on weight reduction and cardiovascular risk. *Lancet* 2005;366:367–8.
103. Michaelsen A, Lehmann N, Pithan C, et al. Mediterranean diet has no effect on markers of inflammation and metabolic risk in patients with coronary artery disease. *Eur J Clin Nutr* 2006;60:478–85.
104. Erlinger TP, Miller ER III, Charleston J, et al. Inflammation modifies the effects of a reduced-fat low-cholesterol diet on lipids: results from the DASH-sodium trial. *Circulation* 2003;108:150–4.
105. The World Health Report 2004. Global Strategy on diet, Physical Activity, and Health. Geneva: World Health Organization, 2004.
106. Aria E, Anderson RN, Kung HC, et al. Deaths: final data for 2001. *Nat Vital Stat Rep* 2003;52:1–115.
107. Mokdad AH, Ford ES, Bowman BA, et al. Prevalence of obesity, diabetes and obesity-related health risk factors, 2001. *JAMA* 2003;289:76–9.
108. Flegal KM, Graubard BI, Williamson DF, Gail MH. Excess deaths associated with underweight, overweight, and obesity. *JAMA* 2005;293:1861–7.

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