

n-3 Fatty acids and the metabolic syndrome¹⁻⁴

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ABSTRACT

The metabolic syndrome is defined as the coexistence of 3 or more components, some of which indicate alterations in glucose and lipid metabolism. The prevalence of the metabolic syndrome is rapidly increasing in relation to obesity, and it is considered to be an important predictor of cardiovascular disease. Increased intakes or supplements of n-3 marine fatty acids may improve defects in insulin signaling and prevent alterations in glucose homeostasis and the further development of type 2 diabetes. This is largely mediated through a reduction in fatty acid accumulation in muscle and liver. n-3 Polyunsaturated fatty acids (n-3 PUFAs) reduce plasma triacylglycerols and improve the lipoprotein profile by decreasing the fraction of atherogenic small, dense LDL. However, n-3 PUFAs do not lower LDL cholesterol. These effects are likely mediated through the activity of transcription factors relating to expression of genes involved in lipid oxidation and synthesis. Other pleiotropic effects of n-3 PUFAs may contribute to decreasing the burden of the metabolic syndrome, such as modulating inflammation, platelet activation, endothelial function, and blood pressure. Although studies comparing the effect of both major n-3 PUFAs are limited, docosahexaenoic acid appears at least as efficient as eicosapentaenoic acid in correcting several risk factors. The use of n-3 PUFAs should be considered in more global strategies including changes in lifestyle, such as adhering to a healthy Mediterranean type of diet and practicing regular physical exercise. *Am J Clin Nutr* 2006; 83(suppl):1499S-504S.

KEY WORDS Metabolic syndrome, n-3 fatty acids, glucose homeostasis, insulin resistance, diabetes, lipid metabolism, lipoprotein, cardiovascular prevention

THE METABOLIC SYNDROME

The metabolic syndrome classically refers to the combination of 3 or more different components (1), which have been recently revised (2): 1) abdominal obesity, which for practical reasons is defined as a waist circumference >94 cm in men and 80 cm in women for the white population; 2) a high concentration of serum triacylglycerols (≥ 1.7 mmol/L, or 150 mg/dL) or current treatment for elevated triacylglycerols; 3) a low concentration of HDL cholesterol [< 0.9 mmol/L (40 mg/dL) in men and < 1.1 mmol/L (50 mg/dL) in women] or current treatment for reduced HDL cholesterol; 4) elevated blood pressure ($\geq 130/85$ mm Hg) or current antihypertensive treatment; and 5) high fasting concentrations of plasma glucose (≥ 5.56 mmol/L, or 100 mg/dL) or current treatment for elevated glucose. These values may need to be modified for particular ethnic groups (eg, groups of Asian origin) (2) and to become more restrictive according to recent

reports of an increased risk of type 2 diabetes at glucose concentrations < 100 mg/dL (3).

As shown by the third National Health and Nutrition Examination Survey, the metabolic syndrome (according to the National Cholesterol Education Program criteria) had a prevalence of $\approx 44\%$ among the US population over 50 y of age in 2003 (4); this prevalence is expected to increase in the near future because of the current epidemic of obesity. It may already exceed 50% of individuals aged > 50 y, and involves an increased proportion of younger subjects. Of particular relevance, the metabolic syndrome represents a significant univariate predictor of coronary heart disease prevalence and is associated with a cardiovascular risk that exceeds the sum of risks corresponding to each individual component (5); this holds particularly true in subjects with type 2 diabetes (6). Indeed, the metabolic syndrome is frequently associated with alterations in composition and function in key organs, as well as with inflammatory conditions and oxidative stress.

Whereas 85% of persons with type 2 diabetes are obese, "only" 30% of obese subjects are diabetics. The mechanisms responsible for the onset of the metabolic syndrome have not been totally clarified. They certainly involve a combination of genetic and behavioral factors, but whether they primarily induce insulin resistance, which in turn results in impaired glucose metabolism and dyslipidemia, or whether lipid alterations come first and then lead to insulin resistance and impaired glucose metabolism has not been firmly established (7). Several features of the metabolic syndrome may be improved by nutritional manipulations, including increased dietary intakes of n-3 long-chain polyunsaturated fatty acids (n-3 PUFAs).

IMPAIRED GLUCOSE TOLERANCE AND INSULIN RESISTANCE

Insulin resistance corresponds to a decreased efficacy of insulin to stimulate glucose uptake in skeletal muscles and adipose

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tissues. In the liver of insulin-resistant animals, glucose-6-phosphatase is overexpressed and hepatic glucose output is less efficiently inhibited by insulin. In the first stage, the combination of these factors leads to increased insulin secretion; in more advanced stages, these factors lead to alterations in glucose homeostasis and type 2 diabetes.

The frequent association of visceral obesity with high concentrations of plasma free fatty acids has led to the concept of an imbalance between the oxidation of fat and of glucose, as described by Randle (8), to be a primary determinant of insulin resistance. However, fat deposition in the liver and also in muscle (9–11) suggests poor regulation between fat delivery and oxidation in these organs.

Impairments in glucose metabolism are associated with molecular alterations of insulin signaling, which are particularly well characterized in muscle (12). An increased content of fatty acids (and their metabolites) appears to favor serine phosphorylation of insulin receptor substrate 1 (IRS-1), and to block IRS-1 tyrosine phosphorylation and the associated activation of phosphatidylinositol-3' kinase activity. This results in a decreased translocation of the glucose transporter GLUT4 to muscle membranes (13). A similar mechanism involving IRS-2 is suggested to occur in liver (14). Defects in mitochondrial fatty acid oxidation and in adipocyte fat metabolism may increase fatty acid content in muscle and liver and precipitate insulin resistance. Thus, impaired transport of glucose and defective glycogen synthesis in muscle, together with sustained hepatic output of glucose seem to mainly result from ectopic fat deposition.

Fat deposition in liver and other organs (eg, muscle and endocrine pancreas) suggests additional causative disturbances, such as increased fatty acid synthesis and reduced fat oxidation during overnight fasting and increased fatty acid mobilization from adipose stores during postprandial hyperinsulinemia. In addition, oxidative stress and cytokine induction appear to participate in the development of nonalcoholic steatohepatitis (15).

Nutritional modulation and the level of physical exercise may affect insulin resistance and the occurrence of the metabolic syndrome in man (16); appropriate changes in diet and more generally in lifestyle may be particularly relevant in subjects with impaired glucose tolerance. High-fat diets, particularly those rich in saturated fats, adversely affect insulin action and may alter HOMA (homeostasis model assessment) values within 60 h when associated with physical inactivity in healthy human volunteers (17). In contrast, supply of n–3 PUFAs rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) to rats fed a high-fat diet totally prevents insulin resistance in muscle (by decreasing fat content and maintaining normal phosphatidylinositol-3' kinase activity and expression and translocation of GLUT4 receptors) and in liver (by maintaining the inhibition of hepatic glucose production) (18).

In healthy humans, a 3-wk supplementation with fish oil (1.1 g EPA + 0.7 g DHA/d) decreased the insulin response to an oral glucose load by $\approx 40\%$. n–3 PUFA dietary enrichment resulted in lower glucose oxidation, higher fat oxidation, and increased glycogen storage; the glycemic response was unchanged, however, which indicates an improved sensitivity to insulin (19).

Thus, n–3 PUFAs present in fish oil may provide a valuable nutritional tool for preventing or diminishing muscular insulin resistance associated with obesity; however, they do not restore insulin sensitivity in the liver (20) and appear to be no longer

efficacious once type 2 diabetes is established (21). Supplementation of n–3 PUFAs to premenopausal, nondiabetic female subjects was reported to markedly decrease the insulin response to an oral glucose load in those with a high level of inflammatory indexes, whereas the effect was less and not significant in those with low inflammatory status (22). On the basis of *in vitro* studies, concerns have also been raised about the possibility of n–3 PUFAs inhibiting pancreatic insulin secretion (20); this needs to be thoroughly assessed *in vivo* in humans. It should be noted that adverse effects have been reported for n–3 PUFAs used in patients with type 2 diabetes: supplementation with both EPA and DHA transiently raised fasting glucose concentrations in diabetic patients with treated hypertension (23).

Supplementation with DHA has been shown to reduce aggressiveness in Japanese students during examination periods (24), and supplementation with fish oil also blunts the sympathetic activity elicited by mental stress in healthy volunteers (25). This observed decrease in adrenal response may also contribute to protection against insulin resistance by controlling the release of counterregulatory hormones such as cortisol.

ALTERATIONS OF LIPID METABOLISM

Classical cardiovascular disease risk factors such as total cholesterol and LDL-cholesterol concentrations are not included as components of the metabolic syndrome. In contrast, other atherogenic factors such as hypertriglyceridemia and low HDL cholesterol are considered. This is in agreement with recent concepts that qualitative modifications may have a greater affect on cardiovascular disease risk than changes in specific lipid concentrations. An increased subfraction of small, dense LDL is commonly found in subjects with the metabolic syndrome together with low concentrations of HDL particles. Apolipoprotein B (apo B) is associated with atherogenic lipoproteins [one apo B-100 molecule per VLDL, IDL (intermediate density lipoprotein), and LDL particle], whereas apo A-1 is present largely in an atheroprotective particle (with 2 to 4 apo A-1 molecules per HDL particle). Therefore, recent publications recommend monitoring the concentrations of both these apoproteins and their ratio in patients with the metabolic syndrome (26, 27).

Cholesterol metabolism is modulated by body weight, as shown by a study in persons with non-insulin-dependent type 2 diabetes (28). In this population, increases in body mass index, which were often linked to higher serum insulin or glucose concentrations, were related to higher rates of cholesterol synthesis and turnover. This was associated with compensatory mechanisms such as a higher cholesterol elimination in bile, an increased conversion into bile acids, and a lower intestinal absorption (28). Mechanisms responsible for the major disturbances of lipid metabolism observed in patients with the metabolic syndrome are illustrated in **Figure 1A**. The high lipolytic rate in visceral adipose depots provides the liver with large amounts of free fatty acids; high glucose and insulin concentrations increase lipogenesis, whereas impaired fat oxidation stimulates fatty acid esterification into triacylglycerols; this, together with an augmented synthesis of apo B-100 and of cholesterol, increases the formation and secretion of VLDL; and insulin concentrations maintain a high lipoprotein lipase activity in adipose tissues and a high rate of VLDL conversion into IDL and LDL. Cholesterol ester transfer protein-mediated exchanges of triacylglycerols and cholesteryl esters are activated by the high concentration of

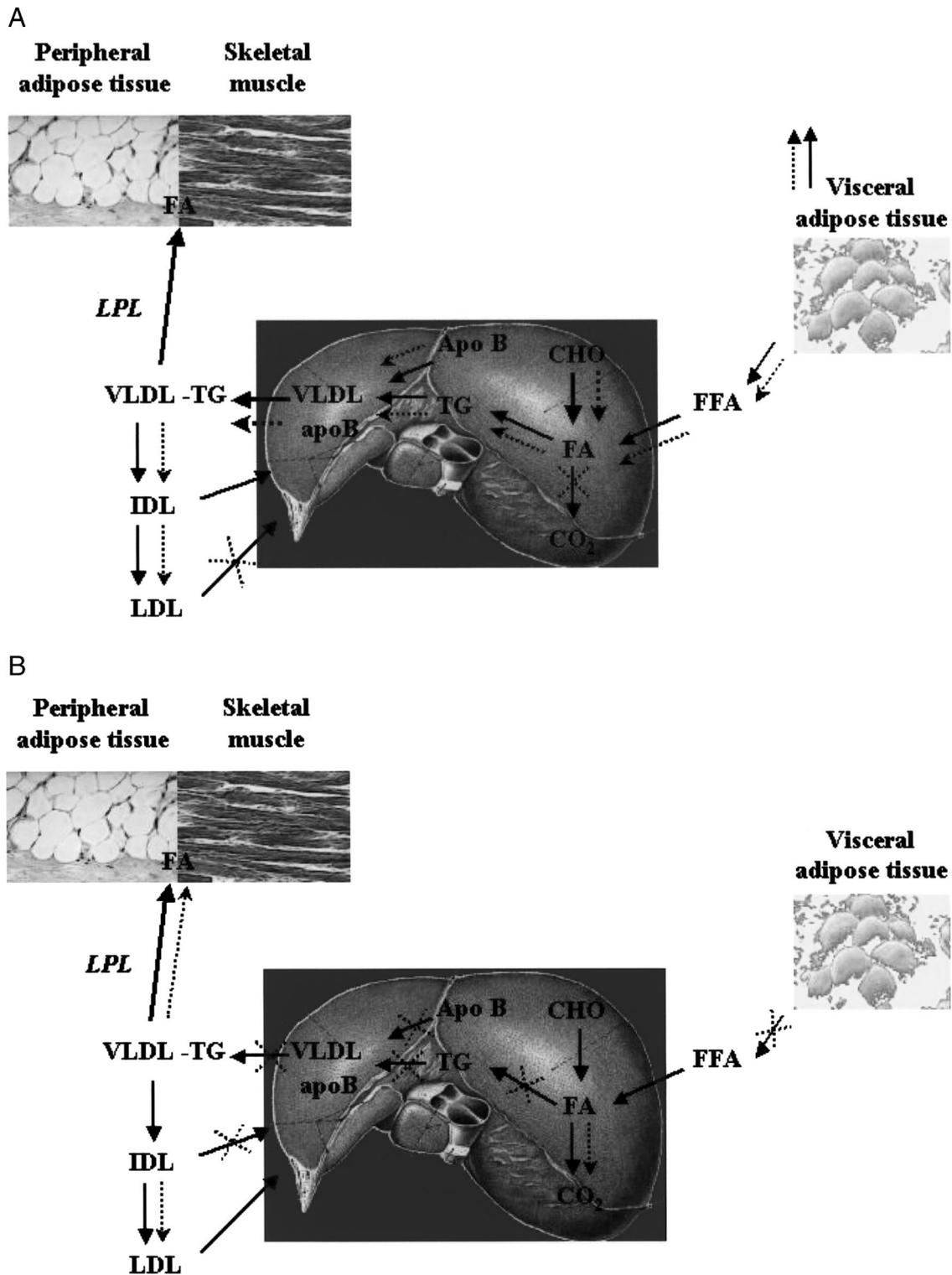


FIGURE 1. A) Common alterations in lipid and lipoprotein metabolism in patients with metabolic syndrome (*see* text for details). apo, apolipoprotein; CHO, carbohydrate; FA, fatty acid; FFA, free fatty acid; IDL, intermediate-density lipoprotein; LPL, lipoprotein lipase; TG, triacylglycerol. B) Supplementation with n-3 polyunsaturated fatty acids: effects on lipid and lipoprotein metabolism (*see* text for details).

triacylglycerol-rich particles, which leads to the formation of small, dense LDL, namely in subjects with a genetic predisposition to the so-called B phenotype. There is an impaired recognition of small, dense LDL by LDL receptors, which maintains

high LDL-cholesterol concentrations in the circulation (designed from reference 29).

Supplementation with n-3 PUFAs favorably modifies many adverse serum and tissue lipid alterations related to the metabolic



syndrome (Figure 1B). The most consistent finding is a drastic reduction in fasting and postprandial serum triacylglycerols and free fatty acids (30). This has been observed with EPA and DHA alone (23) and with their combination in fish oil; multivariate analyses suggest EPA enrichment in platelet phospholipids to be independently associated with serum triacylglycerol lowering (31). Reduced VLDL production in the liver (32) largely results from a decreased availability of free fatty acids released from adipose stores, together with the suppression of lipogenic genes and the induction of genes involved in fatty acid oxidation (33). This regulation of gene expression proceeds through the inhibition of sterol response element binding protein 1 and the activation of peroxisome proliferator-activated receptor (34). Net production of apo B is also reduced. An increased lipolytic activity of lipoprotein lipase in extrahepatic tissues completes the hypotriglyceridemic effect of n-3 PUFAs. n-3 PUFAs have contrasting effects on LDL, with a general tendency toward slightly increased LDL-cholesterol concentrations; however, the potential associated cardiovascular risk is largely compensated for by a reduction in the fraction of atherogenic small, dense lipoprotein particles. In addition, triacylglycerol lowering also affects cholesteryl ester transfer protein-mediated exchanges, which results in (generally modest) increases in HDL cholesterol and possibly apo A-I concentrations.

n-3 PUFA-induced changes in cellular fatty acid partitioning, away from triacylglycerol synthesis pathways and toward fat oxidation, may have favorable effects in subjects with the metabolic syndrome by reducing ectopic fat deposition and associated organ lipotoxicity. Other recognized benefits of n-3 PUFAs consist of a reduction in inflammatory status, decreased platelet activation, mild reduction in blood pressure, improved endothelial function, and increased cellular antioxidant defense, all of which may prove particularly favorable in overweight, hypertensive patients (35).

Some of the effects of n-3 PUFAs on lipid and lipoprotein metabolism could remain in subjects who become overtly diabetic. However, cardiovascular risk prevention may be optimized by the association of n-3 PUFA supplementation with other components of lifestyle, ie, weight control, regular physical activity, and consumption of other dietary ingredients contributing to risk reduction (36).

In this respect, the beneficial effects of a Mediterranean diet, especially when combined with lower energy intakes and increased levels of physical activity, should not be neglected. In a recent study in premenopausal obese subjects with no evidence of diabetes, hypertension, or hyperlipidemia, such adaptations in lifestyles significantly reduced concentrations of interleukin 6 and 18 and of C-reactive protein, while raising adiponectin concentrations (37).

In a substantial proportion of patients with mixed hyperlipidemia, the benefits of n-3 PUFAs may be additive to those of lipid-lowering therapy, with cumulative and complementary effects on lipoprotein metabolism. In a recent study assessing changes in apo B kinetics in insulin-resistant obese subjects, an n-3 PUFA-rich preparation (Omacor, Pronova Biocare, Oslo, Norway) lowered plasma VLDL-apo B concentrations by reducing secretion and activating VLDL conversion into LDL; atorvastatin (40 mg/d) lowered plasma concentrations of all apo B-containing lipoproteins by enhancing the fractional catabolic rate of apo B in VLDL, IDL, and LDL (38). Of related interest,

different lipid-lowering therapies were recently suggested to alter essential fatty acid profiles in patients with coronary heart disease (39).

EPA, DHA, OR OTHER n-3 PUFAS?

Epidemiologic observations of the beneficial properties of n-3 PUFAs have been made in populations consuming large amounts of fatty fish and marine mammal oils (40). Most subsequent studies confirming these effects and analyzing the involved mechanisms used fish oils or a combination of EPA and DHA. Although diets with an increased content of plant α -linolenic acid (18:3n-3, or ALA) appear largely beneficial in secondary cardiovascular prevention (41, 42), it is difficult to determine whether ALA has a direct effect on glucose or lipid homeostasis, on inflammation, or on antioxidant status. Therefore, ALA may exert cardioprotection via an antiarrhythmic effect (43) or by its limited conversion to EPA. In mildly hyperlipidemic subjects, supplementation with EPA and DHA (1.7 g/d) was more efficient in reducing plasma triacylglycerol concentrations than a >5 times larger intake of ALA (9.5 g/d) but seemed to increase LDL susceptibility to oxidation (44). Still, an increased ALA intake may better balance the ratio of n-3 to n-6 fatty acids in subjects eating very low amounts of fish or none at all (45).

Although the key role of DHA has long been recognized in the development and maturation of the fetal central nervous system and retina, metabolic, antiinflammatory and cardioprotective effects of fish oils were primarily attributed to EPA (40). Few controlled studies have compared purified preparations of EPA and DHA to assess the independent effects of these PUFAs. Grimsgaard et al (46) reported on the effects of supplementation with highly purified EPA (3.8 g/d) or DHA (3.6 g/d) for 7 wk in healthy, nonsmoking male volunteers. They found a reduction in plasma triacylglycerols that was at least as marked in the DHA group (-26%) as in the EPA group (-21%); in addition, HDL cholesterol increased only in the DHA group, whereas apo A-I decreased in the EPA group (46). A second study (using dietary supplementation with 4 g/d of ester concentrates of either EPA or DHA) showed that heart rate decreased only in the DHA group, the change being related to the concentration of serum phospholipid DHA and docosapentaenoic acid (47). These results were recently confirmed in a series of studies by Mori and Woodman (reviewed in reference 35) that compared the effect of a high intake (4 g/d) of EPA versus DHA in overweight, hypercholesterolemic subjects and in treated hypertensive subjects with type 2 diabetes. The results provide convincing evidence that EPA and DHA are equally effective at reducing serum triacylglycerols, but that DHA only may raise HDL cholesterol (specifically the HDL₂ fraction) as well as LDL particle size, ie, both antiatherogenic outcomes. In addition, DHA also appeared to be more efficient in lowering blood pressure, improving endothelial relaxation, and attenuating vascular constriction. In contrast, similar reductions of oxidative stress and cytokine production were obtained with EPA and DHA. Although EPA has long been considered as the major n-3 PUFA involved in the prevention of metabolic alterations and cardiovascular diseases, recent studies suggest that DHA may have specific protective properties (Table 1). These results need to be confirmed by other groups, and the effects of other (lower) intakes of n-3 PUFAs should also be evaluated.

TABLE 1

Effects of n-3 fatty acids on different components of the metabolic syndrome

Insulin resistance and glucose homeostasis
↓ Insulin resistance in muscle > adipose tissue >> liver (18)
Possible ↓ insulin secretion (17)
May prevent occurrence of type 2 diabetes but does not reverse it (18)
Lipid and lipoprotein profile
↓ ↓ Serum triacylglycerols (43)
↑ HDL cholesterol (modest)
Possible ↑ LDL cholesterol
↓ Small, dense LDL fraction
Improved profile in mixed hyperlipidemia (without and with type 2 diabetes)
Reduced blood pressure (moderate)
Improved endothelial function
Improved inflammatory status and cell antioxidant defenses

CONCLUSIONS

Supplementation with marine n-3 PUFAs may be indicated in patients with the metabolic syndrome. Such supplementation may contribute to reducing insulin resistance in muscle and diminishing the risk of type 2 diabetes. A decreased ectopic accumulation of fatty acids in muscle and liver appears to be a major component of the mechanisms involved. n-3 PUFAs may also improve the plasma lipoprotein profile by decreasing triacylglycerol concentrations and, even in the absence of LDL lowering, may reduce the fraction of atherogenic small, dense LDL. Pleiotropic effects of n-3 PUFAs include antiinflammatory and antithrombotic responses with a decrease in platelet and leukocyte activation, improved endothelial function, arterial compliance, and blood pressure control. Such supplementation should be integrated into a more global strategy that includes focus on other components of healthy lifestyle (weight control, smoking cessation, regular physical activity, and an adaptation of the Mediterranean diet) and on tight control of LDL cholesterol when indicated. 

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