Available online at www.sciencedirect.com

SCIENCE DIRECT

Journal of Nutritional Biochemistry 17 (2006) 1–13

Journal of Nutritional Biochemistry

REVIEWS: CURRENT TOPICS

Effects of dietary polyunsaturated n-3 fatty acids on dyslipidemia and insulin resistance in rodents and humans. A review

Yolanda B. Lombardo*, Adriana G. Chicco

Department of Biochemistry, School of Biochemistry, University of Litoral, Santa Fe 3000, Argentina

Abstract

For many years, clinical and animal studies on polyunsaturated n-3 fatty acids (PUFAs), especially those from marine oil, eicosapentaenoic acid (20:5,n-3) and docosahexaenoic acid (22:6,n-3), have reported the impact of their beneficial effects on both health and diseases. Among other things, they regulate lipid levels, cardiovascular and immune functions as well as insulin action. Polyunsaturated fatty acids are vital components of the phospholipids of membrane cells and serve as important mediators of the nuclear events governing the specific gene expression involved in lipid and glucose metabolism and adipogenesis. Besides, dietary n-3 PUFAs seem to play an important protecting role against the adverse symptoms of the Plurimetabolic syndrome. This review highlights some recent advances in the understanding of metabolic and molecular mechanisms concerning the effect of dietary PUFAs (fish oil) and focuses on the prevention and/or improvement of dyslipidemia, insulin resistance, impaired glucose homeostasis, diabetes and obesity in experimental animal models, with some extension to humans.

Keywords: Dietary fish oil; Dyslipidemia; Insulin resistance; Diabetes; Obesity; Sucrose-rich; High-fat diets

1. Introduction

For over two decades, numerous studies and clinical investigations have focused on the metabolism of polyunsaturated fatty acids (PUFAs). Most of them place considerable interest in the dietary intake of marine PUFAs, especially eicosapentaenoic acid (EPA) (20:5,n-3) and docosahexaenoic acid (DHA) (22:6,n-3), which are abundant in fish, shellfish and sea mammals and scarce or absent in land animals and plants [1]. Their results give evidence of the beneficial effects of these acids on both normal health and chronic diseases, such as regulation of lipid levels [2,3], cardiovascular [4,5] and immuno functions [6]. In addition, they are essential for normal growth and development. Docosahexaenoic acid (22:6,n-3) is a vital component of the phospholipids of cellular membranes, especially in the brain and the retina, necessary for their proper functioning [7]. Besides, n-3 fatty acids have antiinflammatory, antithrombotic, antiarrhythmic and vasodilatory properties, some of these effects being modulated through prostaglandins and leukotriene metabolism [5–8].

Another important action of n-3 fatty acids is that they could play a key role in the prevention and management of several diseases such as coronary heart disease, dyslipidemia, type 2 diabetes, insulin resistance, hypertension and so on [9–11]. When added to the diet, the EPA and DHA (PUFAs) present in fish or fish oil can alter the membrane phospholipid composition of the cells, impact eicosanoid synthesis and action, and regulate transcription factor activity and abundance. Recent studies suggest that n-3 fatty acids serve as important mediators of gene expression,

Abbreviations: ACC, acetyl-CoA carboxylase; AOX, acyl-CoA oxidase; Apo A-1, apolipoprotein A-1; Apo B, apolipoprotein B; CPT1 and CPT2, carnitine palmitoyltransferase 1 and 2; CYP4A2, cytochrome P450 4 A2; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FAO, peroxisomal fatty acid oxidase; FAS, fatty acid synthase; FFA, free fatty acids; GIR, glucose infusion rate; Glut 4 and 2, glucose transporter 4 and 2; HDL-C, high-density lipoprotein cholesterol; HSL, hormone-sensitive lipase; IRS-1 and IRS-2, insulin receptor substrate-1 and 2; LDL-C, low density lipoprotein cholesterol; LDL receptor, low-density lipoprotein receptor; L-PK, L-pyruvate kinase; LPL, lipoprotein lipase; PDHc, pyruvate dehydrogenase complex; PDH-kinase, pyruvate dehydrogenase kinase; PEPCK, phosphoenolpyruvate carboxykinase; PI3′kinase, phosphatidylinositol 3-kinase; PPAR, peroxisome proliferator-activated receptor; PUFAs, polyunsaturated fatty acids; S14, S14 protein; SCD1, stearoyl-CoA desaturase-1; SREBP-1, sterol regulatory element-binding protein-1; UCP1, uncoupling protein 1; VLDL, very low-density lipoprotein.

* Corresponding author.

E-mail address: ylombard@fbcb.unl.edu.ar (Y.B. Lombardo).

0955-2863/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2005.08.002

working via the peroxisome proliferator-activated receptors (PPARs) controlling the expression of the genes involved in lipid and glucose metabolism and adipogenesis [7]. Moreover, experimental studies have shown that fish oil could down-regulate the hepatic mRNA level of the sterol regulatory element-binding protein-1 (SREBP-1), which also controls several lipogenic genes [12–14].

This review highlights some recent advances in the understanding of metabolic and molecular mechanisms concerning the effect of dietary PUFAs (fish oil) and focuses on the prevention and/or improvement of dyslipidemia, insulin resistance, impaired glucose homeostasis, diabetes and obesity in experimental animal models, with some extension to humans.

2. Effect of fish oil on dyslipidemia, insulin resistance, impaired glucose tolerance, diabetes and obesity

2.1. Studies in experimental animal models

2.1.1. Dietary fish oil prevents the development of dyslipidemia, impaired glucose tolerance, insulin resistance and obesity

2.1.1.1. High sucrose- or fructose-fed rodents. Numerous investigations including our own [15–28] have demonstrated that normal rats fed high carbohydrate (particularly fructose or sucrose) diets for a short period (3–5 weeks) develop hypertriglyceridemia, increased plasma free fatty acid (FFA) levels, enhanced triglyceride accumulation in liver and in some peripheral tissues (e.g., skeletal and heart muscle), hyperinsulinemia, insulin resistance in target tissues (e.g., liver, skeletal muscle, adipose tissue), moderate adiposity and hypertension. Interestingly, several of these metabolic abnormalities are also present in the so-called Plurimetabolic syndrome or Syndrome X in humans [29]. However, numerous studies have shown that when fish oil replaces the usual oil present in the rats' diet as a main source of fat in the sucrose-rich diet, it prevents the onset of dyslipidemia and hyperinsulinemia.

2.1.1.1.1. Effects on the liver. In the hypertriglyceridemic insulin-resistant, sucrose-fed rat model, dietary fish oil is associated with a number of effects that collectively act to reduce dyslipidemia and improve insulin action [30–32]. Fish oil decreases plasma and liver triglyceride levels and very low-density lipoprotein triglyceride (VLDL-TG) secretion, and suppresses postprandial hypertriglyceridemia [33]. This is probably due to both the suppression of the transcription of gene-encoding lipogenic enzymes and the increased fatty acid oxidation. Studies in vivo and with primary hepatocytes have shown that the lipogenic gene expression is unaffected by feeding monounsaturated fatty acids but is suppressed by n-3 or n-6 PUFA. Dietary n-3 and n-6 families (in rodents and probably in humans) suppress the transcription of hepatic gene encoding for lipogenic enzymes [e.g., acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), S14 protein (S14), stearoyl-CoA desaturase-1 (SCD1)], malic enzyme, ATP citrate lyase and glycolytic enzymes [e.g., L-pyruvate kinase (L-PK)], and more recently, apolipoprotein A-1 and delta 5 and delta 6 desaturase [34–36]. However, n-3, but not n-6, fatty acids suppress triglyceride synthesis, VLDL secretion and lower triglyceride levels [36] by mechanisms that include impaired VLDL assembly and secretion [37], direct suppression of VLDL apolipoprotein B (Apo B) by EPA and the like [38].

Halvorsen et al. [39] demonstrated that the administration of fish oil (20:5,n-3 and 22:6,n-3) to rats markedly increases the mitochondrial carnitine palmitoyltransferase 2 (CPT2) activity and peroxisomal fatty acid oxidase (FAO). CPT1 also increases with n-3, both in the presence or absence of malonyl-CoA, in the same way as mitochondrial and peroxisomal β oxidation. Polyunsaturated fatty acid diets stimulate both gene expression and activities of the enzymes involved in peroxisomal β oxidation (FAO is increased). The increases of β oxidation may direct FFA away from triglyceride synthesis with a concomitant decrease of triglyceride secretion, both contributing to the hypolipemic effect of fish oil.

Neschen et al. [40] have recently shown that dietary fish oil (n-3) administered to rats increases the fatty acid capacity of their liver through its ability to function as ligand activator to PPAR-α, and thereby induces the transcription of several gene-encoding proteins affiliated with fatty acid oxidation. 20:5,n-3 CoA compared to 18:1 CoA is a poor substrate for diacylglycerol acyltransferase [41]. Therefore, it decreases the rate of 20:5 n-3 assimilation to neutral lipids and might lead to an elevation of intracellular 20:5 n-3 sufficient to activate PPAR-α [7]. Peroxisome proliferator-activated receptor α is required for the fish oil-mediated induction of mRNA acyl-CoA oxidase (AOX) and mRNA cytochrome P450 4 A2 (CYP4A2), but not for the fish oil-mediated suppression of mRNA encoding FAS, S14 or L-PK [42,43].

Moreover, a recent study examining fish oil feeding on several gene expressions of PPAR knockout mice clearly indicates that hepatic gene regulation by fish oil feeding involves at least two different pathways: PPAR-α dependent and PPAR-α independent [42]. Enzymes for peroxisomal (CYP4A2) and microsomal (AOX) oxidation are PPAR-α dependent and up-regulated, whereas enzymes for lipid synthesis (FAS, S14) are PPAR-α independent and down-regulated [43]. This indicates that the fatty acid regulation of hepatic de novo lipogenesis and fatty acid oxidation are not mediated through a common factor (e.g., PPAR-α).

Kim et al. [12], feeding C57BL/6J mice on either safflower or fish oil during 5 months, demonstrated that fish oil down-regulates the hepatic mRNA level of the SREBP-1. Compared with dietary safflower, fish oil decreases liver SREBP-1c mRNA but does not alter SREBP-1a mRNA (one of the three forms of SREBPs expressed in the liver). Consistent with the decrease of mature SREBP-1, fish oil feeding down-regulates the

expression of liver SRE-dependent genes such as low-density lipoprotein (LDL) receptor, 3-hydroxy-methyl glutaryl-CoA reductase, 3-hydroxy-methyl glutaryl-CoA synthase, ACC and SCD1. These data suggest that fish oil down-regulates the mature form of SREBP-1 in the liver by decreasing the SREBP-1 mRNA expression, with the corresponding decrease of both mRNAs of cholesterogenic and lipogenic enzymes.

2.1.1.1.2. Effects on the adipose tissue. Substitution of fish oil with standard vegetable oil in insulin-resistant, sucrose-fed rats protected these animals from visceral fat hypertrophy, hypertriglyceridemia and hyperglycemia. Peyron-Caso et al. [44] demonstrated that adipocytes of fish oil-fed rats showed an enhanced lipolysis. Moreover, adipose tissue lipoprotein lipase (LPL) activity was increased 2.2-fold, whereas the FAS activity was markedly lower in the liver but not in adipose tissues. These results suggest that the decrease in visceral fat in fish oil-fed rats could be attributed to a decreased plasma triglyceride concentration and/or increased lipid mobilization rather than to reduced lipid storage. Moreover, Peyron-Caso et al. [32] demonstrated that the adipocytes of rats fed a sucrose-rich diet with fish oil are smaller and more sensitive to insulin stimulation than those obtained from rats fed a sucrose-rich diet with vegetable oil. An increase of plasma leptin levels was also observed in insulin-resistant, sucrose-fed rats after oil administration [45]. However, other investigations reported that fish oil feeding might reduce the leptin gene expression [46].

2.1.1.1.3. Effects on insulin sensitivity. Fish oil decreases the skeletal muscle triglyceride content, improves insulin action on glucose utilization and/or storage in insulin main target tissues and prevents the development of whole-body insulin resistance [30,31].

The mechanism responsible for fish oil-induced prevention of insulin resistance in sucrose-fed rats is still unclear, but several studies have demonstrated a strong association between elevated triglyceride concentration (plasma and/or tissues) and diet-induced insulin resistance. Sucrose-induced hepatic insulin resistance is significantly correlated with hepatic triglyceride content, whereas peripheral insulin resistance is significantly correlated with plasma triglyceride levels [19,47]. An inverse correlation between insulin-stimulated glycogen synthase activity and triglyceride content in the soleus muscle was observed by Klimes et al. [30] in sucrose-fed rats. Thus, one explanation for the prevention of sucrose-induced insulin resistance may be related to the hypolipidemic actions of fish oil.

In addition to the regulation of plasma lipid levels, increasing evidence suggests that the fatty acid composition of the membrane phospholipids of insulin target tissues [48,49], such as liver, fat pad and skeletal muscle, is a critical factor that influences both the insulin secretion and its biological actions (e.g., changes in membrane fluidity, diacylglycerol second messenger function, etc.). Luo et al. [50] demonstrated that insulin action is positively correlated with the fatty acid insaturation index in membrane phospholipids of rat adipocytes when fish oil represents the main source of lipids in the sucrose-rich diet. Moreover, fish oil prevents the decrease of insulin-stimulated glucose transport and the oxidation and incorporation into total lipids. Improved in vitro insulin-stimulated glucose incorporation into total lipids and glucose oxidation into CO_2 were also observed in adipocytes of neonatal streptozotocin diabetic rats fed a sucrose-rich diet to which 30% dietary fish oil had been added [51].

Peyron-Caso et al. [32] observed an increase of insulin-stimulated glucose transport in adipocytes associated with an increase of glucose transporter 4 (Glut 4) protein and mRNA levels in rats fed a sucrose-rich diet supplemented with fish oil, with no changes in plasma glucose levels and no effect on muscle Glut 4 protein levels. Sebokova et al. [52] showed that a raised dietary intake of fish oil in hereditary hypertriglyceridemic rats does not alter the number of Glut 4 protein levels in muscle. Mori et al. [53], in Otsuka Long–Evans Tokushima fatty rats, a model of spontaneous type 2 diabetes mellitus with obesity, demonstrated that the long-term administration of fish oil for 17 to 18 weeks prevents the development of insulin resistance. This seems to depend on a significant increase of the Glut 4 mRNA expression in the skeletal muscle mediated by alteration in muscle membrane phospholipid composition.

In brief, when n-3 fish oil replaces the vegetable oil present in the rats' diet, it prevents the onset of dyslipidemia, insulin insensitivity in main target tissues (liver, skeletal muscle, adipose tissue) and whole-body insulin resistance in high sucrose or fructose-fed rodents. A summary of the effects of fish oil as the major source of fat in the sucrose-rich diet is presented in Table 1.

2.1.1.2. High-fat-fed rodents

2.1.1.2.1. Effects on skeletal muscle. Another interesting experimental model of insulin resistance is that induced by high-fat diets [54]. This model has been useful in the search for the mechanism underlying human disorders associated with insulin resistance and obesity. Experimental studies in rodents (rats and mice) have shown that either saturated (lard), monounsaturated (olive oil: n-9) or polyunsaturated (safflower oil: n-6) high-fat diets alter insulin sensitivity (insulin resistance) in target tissues (e.g., liver, skeletal muscle and adipose tissue) associated with glucose intolerance, hyperinsulinemia and obesity [55,56]. In skeletal muscle, high-fat diets decrease insulin-stimulated oxidative and nonoxidative glucose disposal and increase triglyceride, long-chain acyl-CoA and diacylglycerol contents [57–59]. Storlien et al. [55] showed a deterioration of skeletal muscle insulin action under conditions of increased triglyceride and long-chain acyl-CoA contents.

2.1.1.2.2. Insulin signaling and insulin resistance. Defects in insulin signaling in peripheral tissues were reported during a high-fat diet [60]. An impairment in the

Table 1
Main effects of dietary fish oil in the prevention of experimental dyslipidemia and insulin resistance induced by feeding a high refined sugar (HRS) diet[a]

	HRS	HRS+fish oil[b]
Blood pressure	↑ [17,30]	⊥ [30]
Plasma		
Triglycerides (VLDL-TG)	⇑ [15–25,27,28,30,32,50,80]	⊥ [30,33,50]
FFAs	⇑ [15–25,28,30,32,80]	⊥ [30,32]
Glucose	↑/⊥ [15–25,27,28,30,32,50]	⊥ [30]
Insulin	↑ [15–25,30,32]	⊥ [30,32]
Leptin	↓ [45]	↑ [45]
Peripheral insulin resistance	⇑ [18,19,25,47,83]	⊥ [30,31]
Liver		
Lipogenesis	⇑ [15–25]	⊥ [30,33]
VLDL secretion	⇑ [15–25]	⊥ [30,33]
Triglyceride pool size	⇑ [15–25]	⊥ [30,31,33]
Insulin resistance	⇑ [18,19,25]	⊥ [30,48]
Insulin signaling	Altered [26]	Improved [30]
Skeletal muscle		
Lipids (triglyceride, LCACoA)	↑ [19,30,83]	⊥ [30,31]
Insulin resistance	↑ [18,47,83]	⊥ [30]
Insulin signaling	↓ [26]	
Glucose utilization and/or storage	↓ [18,19,30,83]	⊥ [30]
Adipose tissue (white fat)		
Adipocyte cell size	↑/⊥ [32,28]	↓ [32]
Visceral fat	↑/⊥ [25,28,32,45,50]	⊥ [32,50]
Insulin resistance	↑ [25,32,50]	⊥ [32,50]
Insulin-stimulated glucose transport, oxidation and incorporation into total lipid	↓ [27,32,50]	⊥ [30,32,50]

LCACoA, long-chain acyl-CoA; ↑, moderate increase; ⇑, high increase; ↓, decrease; ⊥, prevents (normal levels).
[a] Fructose/glucose.
[b] Vegetable oil partially replaced by fish oil.

early steps of insulin signaling that could involve insulin receptor substrate-1 (IRS-1) tyrosine phosphorylation as well as phosphatidylinositol 3-kinase (PI3′kinase) activity was observed in skeletal muscle [61]. In adipose tissue, a high-fat diet reduced the IRS-1 and IRS-2 proteins. In liver, IRS-1 and IRS-2 proteins and their phosphorylations were not altered, and the PI3′kinase activity associated with IRS-1 and IRS-2 increased. However, the development of insulin resistance in the high-fat feeding model is clearly dependent on the type of dietary fat [54]. Storlien et al. [62] demonstrated that substitution of n-3 fatty acids from fish oil (largely the long-chain highly unsaturated EPA and DHA fatty acids) with the safflower oil diet (rich in n-6 fatty acids) prevents the development of in vivo insulin resistance and improves insulin action in liver and skeletal muscle.

The mechanisms sustaining the protective effect of n-3 PUFAs remain unclear. Those effects could be related to the subsequent changes in fatty acid content of membrane phospholipids of insulin target tissues [63]. In muscle, n-3 PUFAs might improve insulin sensitivity through a relative increase in the insaturation of membrane phospholipids and/or a decrease in the muscle content of triglycerides [56,64,65]. Alterations in membrane composition could affect insulin receptor (IR) and/or IRS-1 and PI3′kinase expression and protein abundance. Recently, Taouis et al. [61] established the clear effect of a high-fat diet enriched in n-3 fatty acids upon insulin signaling. In the liver, insulin-stimulated IR tyrosine phosphorylation, IRS-1 tyrosine phosphorylation and PI3′kinase activity were almost completely abolished in rats fed a high-fat diet enriched with n-3 fatty acids compared to animals fed a chow diet (low fat). This indicates a profound alteration of the early steps of IR signaling in liver. In the muscle IR, IRS-1 tyrosine phosphorylation, PI3′kinase activity and total Glut 4 content were similar to those recorded in control rats fed a chow diet. These results may at least partially explain the recovery of glucose uptake after substitution of n-6 with n-3 PUFAs in a high-fat diet [62]. In adipose tissue, at the level of gene expression, the diet enriched in n-3 fatty acids partially decreased the expression of p85 without any changes in IR, IRS-1, Glut 4 and leptin mRNA. However, despite those positive effects, rats fed with n-3 PUFAs compared to chow-fed rats still showed hyperglycemia and hyperinsulinemia, indicating that liver insulin sensitivity impairment strongly contributes to insulin resistance. These results suggest that fish oil may have a tissue-specific impact in restoring insulin sensitivity and not always prevents insulin resistance.

On the other hand, when examining the mechanism by which fish oil protects against a high-fat diet-induced insulin resistance, Neschen et al. [40] found that in contrast to control and safflower oil-fed rats, fish oil feeding induces a significant increase in the abundance of peroxisomal AOX and 3-ketoacyl-CoA thiolase in liver but does not have a similar effect in muscle. This was paralleled by an almost twofold increase of hepatic peroxisome content and was not associated with an increase in PPAR-α gene expression in liver. These changes were associated with a more than twofold lower hepatic triglyceride/diacylglycerol as well as intramuscular triglyceride/fatty acyl-CoA content. These data indicate that n-3 fatty acids protect against insulin resistance by serving as PPAR-α ligands and thereby induce hepatic but not intramuscular peroxisome proliferator. In turn, an increased hepatic oxidative capacity results in lower hepatic triglyceride/diacylglycerol and intramyocellular triglyceride/fatty acyl-CoA content.

2.1.1.2.3. Effects on adipose tissue. A high-fat diet increases visceral fat accumulation within a few weeks [66]. Several studies indicate a reduction of white fat pad mass and adipocyte size in rats fed a high-fat diet rich in EPA and DHA (fish oil) or 18:3,n-3 (perilla oil) compared to saturated fat (lard) or rich in n-6 PUFA (safflower oil) [67]. A partial substitution of lard with fish oil in a high-fat diet not only prevents the development of hyperlipemia and

obesity but also induces hypotriglyceridemia, hypocholesterolemia and leanness [68].

Studying the mechanisms by which n-3 PUFAs cause less body fat accumulation, Ohinata et al. [69] observed an increase in thermogenesis in the brown adipose tissue. Kawada et al. [70] compared the effect of different fat diets (lard, fish, linseed and perilla oil added to a commercial diet) on the brown adipose tissue uncoupling protein 1 (UCP1) content, showing that the UCP1 content was significantly higher in rats fed a fish oil diet than in those fed a lard diet. More recently, Takahashi and Takashi [71] found increased brown adipose tissue UCP1 mRNA levels in rats fed a high-fat diet. The increases were greater with fats rich in n-3 PUFAs (EPA, DHA, fish oil and perilla oil) than with n-6 PUFA (safflower oil).

The type of dietary fat affects the lipogenic activity not only in the liver but also in the adipose tissue. Benhizia et al. [72] showed that the activity and mRNA levels of FAS in the white adipose tissue is down-regulated by increasing the fat content in the diet, and fish oil compared to lard or corn oil is more effective in decreasing those variables. Raclot et al. [73], studying the retroperitoneal fat tissue of rats fed high-fat diets (20% fat) containing n-3 PUFAs (mainly DHA), reported a decrease in the mRNA levels of several proteins involved in adipose tissue metabolism including: FAS, hormone-sensitive lipase, LPL, phosphoenolpyruvate carboxykinase, CCAAT/enhancer binding protein alpha (C/EBP) and leptin. In contrast, n-3 PUFAs had no effect on gene expression in subcutaneous fat pad, suggesting that n-3 PUFAs affect gene expression in a site-dependent manner in white adipose tissues via possible antiadipogenic effects. High-fat diets increased the gene expression of leptin in the adipose tissue obtained from various fat pads in rats [74,75]. Accordingly, rats fed a high safflower oil diet doubled the leptin mRNA level in the white adipose tissue, whereas no significant increases were detected with high-fat diets rich in n-3 PUFAs [71].

High-fat feeding down-regulates the Glut 4 mRNA in the white adipose tissue [76,77]. However, the decreases are attenuated in diets rich in n-3 PUFAs [71]. Moreover, dietary fish oil stimulates insulin-dependent glucose transport and metabolism in isolated adipocytes and preserves the deleterious effect of a high-fat diet on the capacity of the adipose tissue to utilize glucose [68,78]. It has also been shown that n-3 PUFAs (especially EPA) bind and activate the PPAR-γ2 (PPAR-γ isoform) expression in the white adipose tissue. Peroxisome proliferator-activated receptor γ2 activation results in a coordinated increase of a large number of genes involved in lipogenesis and fatty acid transport, fatty acid storage and fatty acid oxidation in the white adipose tissue. Peroxisome proliferator-activated receptor γ activation remodels the adipose tissue in adult animals, driving the formation of small insulin-sensitive white adipocytes as well as the differentiation of brown adipocytes that have the potential to dissipate excess energy as heat [75,79]. Table 2 presents a summary of the effects produced in a diet by the partial or total substitution of vegetable oils with n-3 fatty acids from fish oil.

Table 2
Main effects of dietary fish oil on insulin resistance obesity induced by a high-fat diet

	High-fat diet[a]	High-fat diet (partially or totally substituted for n-3 fish oil)
Plasma		
Glucose	⇑ [54–56,61,74]	⊥ / ↑ [55,61]
Insulin	⇑ [54–56,61,66]	⊥ / ↑ [55,61]
Peripheral insulin resistance	⇑ [54,56,62,66]	⊥ [55,62]
Liver		
Lipid content (TG)	⇑ [40,72]	⊥ [40,72]
Lipid oxidation		⇑ [40]
Insulin resistance	⇑ [56,62]	⊥ / ↑ [61,62]
Insulin signaling	Altered [61]	Altered [61]
Glucose production	⇑ [56]	⊥ / ↑ [55]
Skeletal muscle		
Lipid content (TG, DAG, LCACoA)	⇑ [40,54,55,57,59,66]	⊥ [40,54,55]
Insulin resistance	⇑ [54,55,57,59,62,66]	⊥ [54,55,62,66]
Insulin signaling	Altered [60,61]	Improved [61]
Insulin-stimulated glucose transport	↓ [56,66]	⊥ [66]
Oxidation and storage		
Adipose tissue (white fat)		
Adipocyte cell size	⇑ [66,67]	⊥ [66,67]
Visceral fat	⇑ [66–68,71,72,78]	⊥ [66–68,71,78]
Lipid content (TG)	⇑ [66,72]	⊥ [66,72]
ob mRNA	⇑ [66,74]	Improved [71]
Insulin resistance	⇑ [68]	Improved [68]
Insulin signaling	Altered [61]	Improved [61]
Insulin-stimulated glucose transport	↓ [64,76–78]	Improved [64,78]

TG, triglyceride; DAG, diacylglycerol; LCACoA, long-chain acyl-CoA; ↑, moderate increase; ⇑, high increase; ↓, decrease; ⊥, prevents (normal levels).

[a] Saturated (lard), monounsaturated (n-9) and (n-6) polyunsaturated

In short, rats fed a high-fat or high-sucrose/fructose diet exhibit impaired insulin action associated with an oversupply of lipids. This increased availability leads to either elevated lipid stored in insulin target tissues (e.g., muscle, liver and adipose) or increased plasma FFA and triglyceride levels. The partial substitution of vegetable oils with n-3 in both high-sucrose/fructose or high-fat diets is associated with a number of effects that collectively act to prevent or ameliorate insulin resistance.

2.1.2. Dietary fish oil improves or reverses dyslipidemia, impaired glucose homeostasis, insulin resistance and adiposity

Most experimental studies examining the relationship between diet and insulin resistance have focused on the development of the impairment, but relatively few have

examined the effectiveness of dietary nutrients (e.g., fish oil) in reversing diet-induced insulin resistance. One possibility is to examine the effect of dietary fish oil in rats fed a sucrose-rich diet for a long time (up to 40 weeks) instead of a short time (3–5 weeks). With this nutritional model, several years ago, our group [80] demonstrated that the metabolic and hormonal milieus change and deteriorate with the length of time on diet.

2.1.2.1. Characterization of rats fed a long-term sucrose-rich diet. Numerous studies carried out in our laboratory [22,28,81–83] showed that in the presence of dyslipidemia, plasma glucose and insulin evolved from normoglycemia and hyperinsulinemia after a short-term (3–5 weeks) consumption of the sucrose-rich diet to moderate hyperglycemia and normoinsulinemia after a long-term (15 weeks) one. Moreover, from then on, when the feeding period was extended up to 40 weeks, a steady-state hypertriglyceridemia and high plasma FFA levels, hyperglycemia, and worsening of whole-body insulin resistance were observed without changes in circulating insulin levels. At this point, the sucrose-rich fed rats were slightly overweight with a higher increase of visceral adiposity [83–85]. The endocrine pancreas showed a significant increase of both islet number and β cell area, as well as changes in the profile of islet cells distribution, without an increase in the pancreatic content of immunoreactive insulin [86]. In addition, the biphasic patterns of glucose-stimulated insulin secretion from perifused islets showed a progressive deterioration during the period the diet was consumed, illustrated by an absence of the first peak with an increase in the second phase of hormone secretion at 30 to 40 weeks [83]. The temporal metabolic changes described may reflect the early start of type 2 diabetes mellitus, because many patients have chronically elevated plasma FFA and triglyceride levels, altered peripheral insulin sensitivity and loss of the first peak of insulin response to glucose.

Thus, the rats fed a long-term sucrose-rich diet seem to be an appropriate experimental model for studying and disclosing the effect of n-3 PUFAs (fish oil) in correcting or improving these abnormalities.

2.1.2.2. Effects on skeletal muscle. Using this experimental model, Lombardo et al. [87] showed that hypertriglyceridemia and glucose intolerance ensuing long-term feeding normal rats with a sucrose-rich diet could be completely reversed mediating no changes in circulating insulin levels by shifting the source of fat in the diet from corn oil (18% total energy) to fish oil (cod liver oil, 16% plus corn oil 2% total energy) during 1 or 2 months. Under similar experimental conditions, D'Alessandro et al. [88] studied the effect of fish oil on triglyceride metabolism, glucose oxidation and glycogen storage during both the basal state and the euglycemic clamp in skeletal muscle. Fish oil reduced to normal levels the higher triglyceride content within the skeletal cells. The decreased capacity for glucose oxidation in the basal state and during the euglycemic clamp—mainly due to an increase of pyruvate dehydrogenase kinase (PDH-kinase) and decrease of PDH complex (PDHc) activities—was completely normalized by the administration of fish oil. Dietary fish oil reversed the impaired insulin-stimulated glycogen storage during the clamp, the whole-body peripheral insulin insensitivity and the hyperglycemia, and returned to normal plasma FFA levels. The hypolipidemic effect of fish oil decreased the availability and oxidation of the lipid fuel within the skeletal muscle and could in turn restore the glucose oxidation and contribute to normalize peripheral insulin sensitivity [89].

An increase of n-3 EPA and DHA in the phospholipids of the gastrocnemius muscle that could influence the biological action of insulin was observed when dietary fish oil was given to insulin-resistant rats [90]. However, another study [31] found that when the sucrose diet containing menhaden oil (6% calories) was given to insulin-resistant rats for 5 weeks, insulin action on the glucose metabolism remained impaired. Differences in the amount of fish oil present in the diet (16% in D'Alessandro et al. [88] vs. 6% of calories in Podolin et al. [31]), as well as the polyunsaturated–saturated ratio between the two fish oils used (1.23 for cod liver oil [88] vs. 0.88 for menhaden oil [31]), may contribute to the differences in the effectiveness of fish oil in reversing insulin insensitivity.

2.1.2.3. Effects on β cell function. It is known that chronic exposure to high levels of FFA impairs glucose-stimulated insulin secretion "in vivo" and "in vitro" and could negatively influence β cell through lipotoxicity leading to β cell dysfunction [91,92]. Furthermore, chronic hyperglycemia has been shown to have a deleterious effect on both insulin secretion and action, a concept termed glucotoxicity [93,94]. Several mechanisms have been proposed that could contribute to the dysfunction of the β cell [for review see [95–97]]. Pighin et al. [90] have recently demonstrated that the increased fat storage and decreased PDHc activity within the β cells are possible mechanisms for mediating, at least in part, the altered insulin secretion under the stimulus of different secretagogues (e.g., glucose, palmitate, L-arginine) from dyslipemic insulin-resistant rats fed a long-term sucrose-rich diet. The inhibition of PDHc limits the conversion of pyruvate derived from glycolysis to acetyl-CoA and diminishes the oxidative glucose metabolism, a signal for insulin secretion and synthesis [89–98]. This finding is consistent with the reversion of these alterations after the administration of fish oil, which completely normalized both fat storage and the PDHc activity within the β cells as well as the insulin secretion patterns stimulated by glucose, and improved the hormone secretion under the stimulus of either palmitate or L-arginine (Fig. 1).

2.1.2.4. Effects on adipose tissue. A long-term sucrose-rich diet develops visceral adiposity and a moderate increase of body weight. Soria et al. [99] showed that the presence of

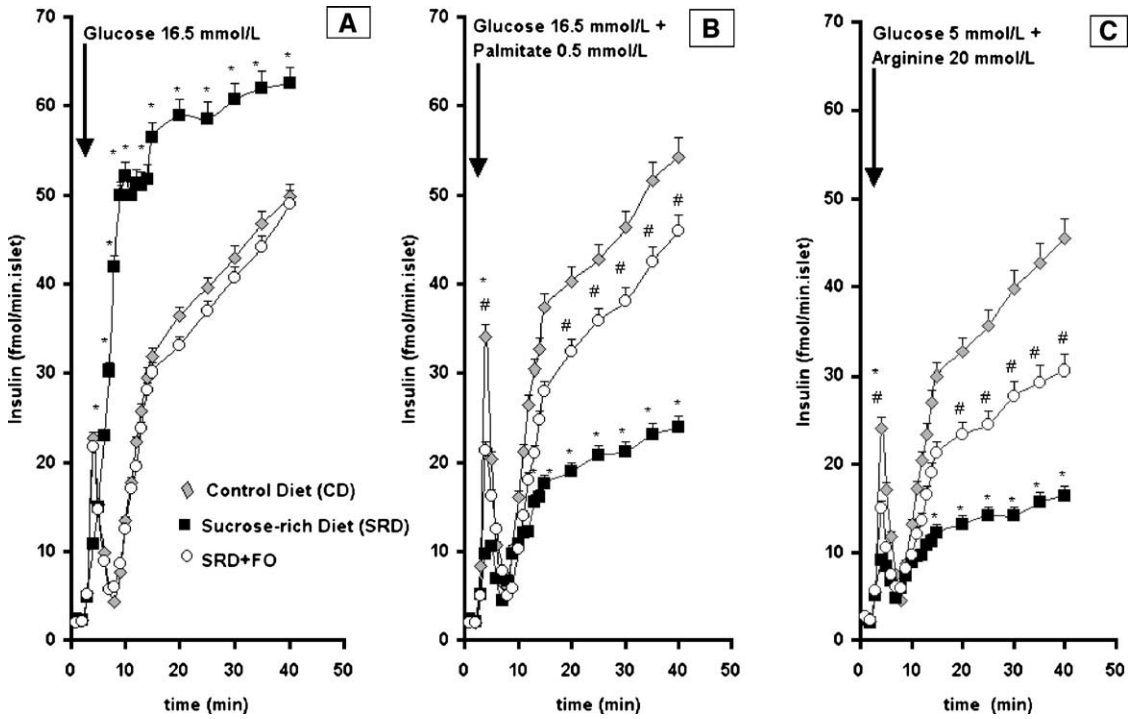

Fig. 1. Insulin secretion in perifused pancreatic islets from rats fed control diet (CD) sucrose-rich diet (SRD) or SRD+fish oil (SRD+FO) diet under the stimulus of glucose (panel A), glucose+palmitate (panel B) or glucose+L-arginine (panel C). Values are mean±S.E.M., $n=6$. *$P<.05$ SRD versus CD and SRD+FO at each time-point in panels A, B and C; #$P<.05$ SRD+FO versus CD at each time-point in panels A, B and C.

dietary fish oil was able to reverse the preexisting metabolic and morphological changes of epididymal fat tissue. Fish oil markedly reduced fat pad mass, the hypertrophy of fat cells, and improved the altered size distribution possibly via mechanisms that included fish oil binding and activation of the PPAR-γ expression in the

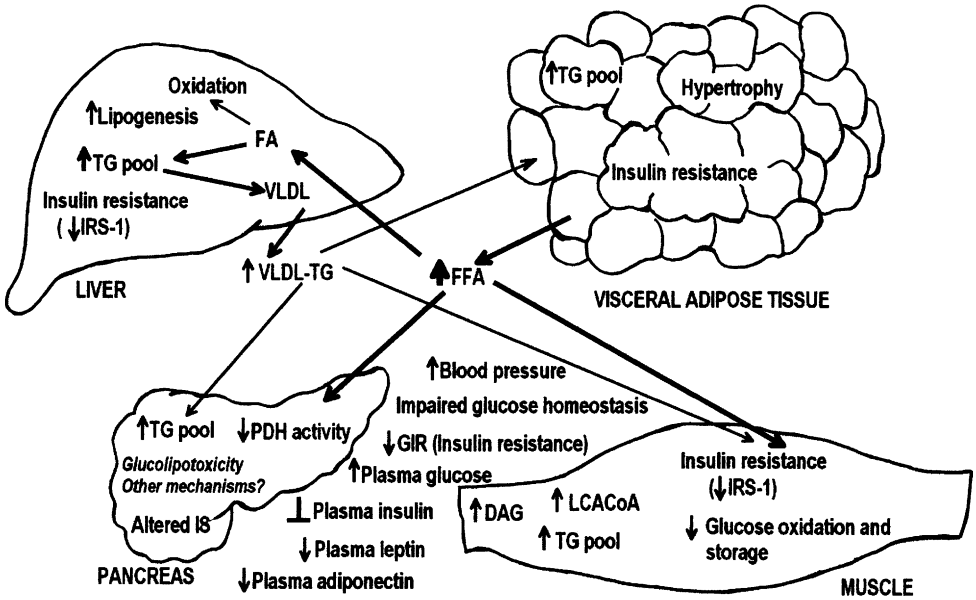

Fig. 2. Effects of long-term sucrose intake on lipid and glucose metabolisms. Chronic intake of a high sucrose diet (up to 40 weeks) induces insulin resistance in liver, adipose tissue and skeletal muscle as well as altered glucose-stimulated insulin secretion from isolated perifused β cell. Hepatic lipogenesis and VLDL-TG secretion are increased. Fatty acids "spillover" from adipose tissue to nonadipose tissue. In liver, the increased flux of plasma fatty acids acts per se as substrate for the hepatic triglyceride pool. Increased fatty acid esterification is relatively favored over oxidation and contributes to the increase of VLDL-TG. The high availability of plasma FFAs increases triglyceride, long-chain acyl-CoA and DAG contents in muscle and enhances the oxidation of lipid fuel. This in turn contributes to the impaired oxidative and nonoxidative glucose pathways associated with insulin resistance. A chronic elevation of both plasma FFA and glucose levels induces an increase of TG pool size while decreasing PDHc activity—a key enzyme in glucose oxidation—within the islets that contribute to the demise of β cells possible through mechanisms involved in the so-called glucolipotoxicity.

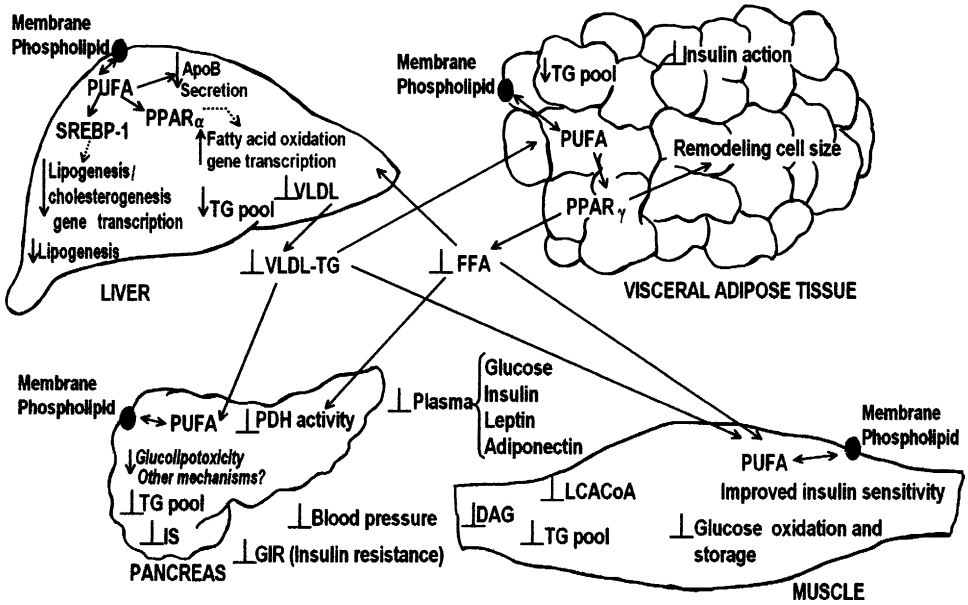

Fig. 3. Possible mechanisms through which the n-3 PUFAs (especially the EPA and DHA derived from fish oil) improve or reverse the metabolic abnormalities induced by the intake of a long-term sucrose-rich diet. Dietary n-3 PUFAs or those released from the membrane phospholipids may exert their effects by up-regulating the expression of genes encoding proteins involved in fatty acid oxidation (through PPARs) while simultaneously down-regulating genes encoding proteins of lipid synthesis (through SREBP-1). The final result of both possible mechanisms in the dyslipemic insulin-resistant rat model is a reduction of hepatic lipogenesis, the TG pool size and VLDL-TG secretion and a normalization of plasma TG levels. In adipose tissue, n-3 PUFAs bind and activate the PPAR-γ expression, a key transcription factor involved in adipogenesis, remodeling adipocyte cell size. The smaller adipocytes are more insulin sensitive and the release of fatty acid is decreased. Therefore, a reduced FFA spillover from adipose tissue to nonadipose tissue is observed. N-3 PUFAs alter the FA composition of membrane phospholipids, and this could influence the insulin biological action. In muscle, dietary n-3 FA normalize lipid contents and restore the nonoxidative and oxidative glucose pathways. In isolated β cells, lipid content and glucose oxidation are normalized. All these effects could contribute to the normalization of glucose-stimulated insulin secretion and muscle insulin insensitivity.

adipose tissue as described above. The presence of fish oil in the diet normalized isoproterenol-stimulated lipolysis and corrected the inhibitory effect of high sucrose upon the antilipolytic action of insulin.

Adipose tissue synthesizes and secretes a large number of biologically active molecules (hormone-like peptides), so-called adipokines (e.g., leptin ,adiponectin, TNF-α, etc.). Both adiponectin and leptin modulate various biological functions and could play an important role in lipid and glucose metabolism [100]. Lombardo et al. [85] showed that the adiposity and insulin resistance present in long-term sucrose feeding was accompanied by a decrease of both plasma leptin and adiponectin levels without changes in the gene expression of visceral fats. By shifting the source of fat to fish oil, the plasma levels of both adipokines were increased, insulin resistance and dyslipidemia were reversed and adiposity improved. Although the mechanisms by which fish oil increased plasma leptin and adiponectin are still unclear, these results suggest that the increase of both adipokines by fish oil might play an essential role in the normalization of insulin resistance and adiposity.

A summary of the effects of fish oil supplementation in the presence of a stable dyslipidemia and insulin resistance achieved by a long-term sucrose-rich diet is depicted in Figs. 2 and 3.

Thus, it is possible that all the mechanisms mentioned above, involving the effects of dietary long-chain n-3 PUFAs acting coordinately, might contribute to prevent, improve or correct dyslipidemia, abnormal glucose homeostasis, insulin resistance and obesity experimentally induced in rodents by feeding them either a high refined sugar or high-fat-rich diet.

2.2. Human studies

Hypertriglyceridemia is often associated with multiple metabolic abnormalities including glucose intolerance, type 2 diabetes mellitus, hyperinsulinemia, insulin resistance, decreased levels of high-density lipoprotein cholesterol (HDL-C), obesity and hypertension (Reaven's Syndrome X or Plurimetabolic syndrome) [29,101].

2.2.1. Nondiabetic patients

Hypertriglyceridemia and elevated low-density lipoprotein cholesterol (LDL-C) and lower HDL-C concentrations are important risk factors for coronary heart diseases [102].

Human studies have shown that fish oil supplements and diets containing fish oil (enriched on EPA and DHA fatty acids) are useful to reduce plasma triglyceride and VLDL lipoprotein concentrations, especially in the postprandial state [5,103]. The mechanism for the reduction of triglyceride rich lipoprotein involves both a decrease in VLDL synthesis [2] and an increase in the fractional catabolic rate of VLDL (triglyceride clearance) [9]. Moreover, a higher

rate of fatty acid oxidation was observed in humans fed PUFAs-rich diets [104].

Concerning middle-aged and elderly patients with increased risk of coronary heart disease, Zhengling et al. [105] have recently shown that in a saturated fat/cholesterol-restricted diet, a high fish oil content favorably affects VLDL and HDL subspecies, shifting lipoproteins subfractions to a less atherogenic pattern. Patients with type IIb or IV hypertriglyceridemia, given a standardized test meal, showed that n-3 fatty acids suppress both hepatic and intestinal Apo B secretion/synthesis and altered triglyceride-rich lipoprotein metabolism (changing postprandial triglyceride, cholesterol, Apo B48 and increasing LDL particle size) [106]. Moreover, regarding blood cholesterol levels and human atherosclerosis, Garcia-Pelayo et al. [107], in studies with Reuber H35 hepatoma cells, showed that 20:5,n-3 down-regulates the HMGCoA reductase without decreased protein synthesis. All these studies could represent additional mechanisms for the reduced heart disease risk associated with n-3 fish oil consumption.

Based on epidemiological findings, the intake of n-3 fatty acids in the diet has been associated with a reduced cardiovascular risk [4]. The mechanisms of risk reduction have been widely explained, either as a consequence of the lipid/lipoprotein changes or as platelet/function and hemorrheological changes, including reduced thromboxane B2 formation and red cell aggregability and viscosity. Monocyte macrophage changes have also been observed, leading to reduced formation of interleukin-1 and platelet aggregation factors, resulting in inhibited growth factor formation and promoting nitric oxide-induced endothelial relaxation [4,5,108]. Furthermore, middle hypotensive and antiarrhythmic effects of n-3 fatty acids have been shown in cell cultures and human studies [1,109]. The antihypertensive effect of n-3 fatty acids may depend on vascular effects with improved endothelial vasodilator function, reduced reactivity of vascular smooth muscle of resistant vessels and increased vascular compliance [1,110].

2.2.2. Diabetic patients

It is known that fish oil intake delays the development of glucose intolerance in humans [111]. Interestingly, a recent work by Stene and Joner [112], in a larger nationwide case-control study in Norway, found a significant association between the use of cod liver oil during the first year of life and a lower risk of type 1 diabetes mellitus, perhaps through the antiinflammatory effects of long-chain n-3 fatty acids.

Concerning the effect of fish oil administration on the glycemic control in diabetic patients with type 2 and type 1 diabetes mellitus, conflicting results have been obtained. Some early studies on type 2 diabetes mellitus [113,114] showed that fish oil may worsen glycemic control; however, the adverse effect of fish oil has almost invariably been established with large doses of omega-3 fatty acids (5.5–8 g/day) in studies lacking an appropriate control group and with a small number of subjects. Other authors found no changes [115,116] or even improved insulin sensitivity in patients with type 2 diabetes mellitus.

A metaanalysis [117] of 26 different trials on the effect of fish oil administration on both glycemic control and lipid parameters in type 2 and type 1 diabetic subjects showed (i) a decrease in plasma triglyceride concentration and slight but significant increase in LDL-C, both findings being more prominent in type 2 diabetes mellitus; no changes in total cholesterol and HDL-C were observed, (ii) no significant changes in HbA_{1c} occurred in diabetic patients, (iii) fasting blood glucose levels increased with borderline significance in type 2 diabetic subjects and were significantly lower in type 1 diabetic subjects; (iv) besides, significantly, dose-response effects of EPA (g/day) on HbA_{1c} and triglycerides and DHA (g/day) on fasting blood glucose levels, HbA_{1c} and triglycerides, were demonstrated only in type 2 diabetic subjects [117].

Recently, Skurnick-Minot et al. [118] demonstrated a decrease in whole-body adiposity and adipocyte size in type 2 diabetic insulin-resistant patients after 2 months of treatment with fish oil capsule (1.8 g of n-3 PUFA). In these patients, plasma adiponectin tended to increase without any deterioration or amelioration of insulin sensitivity at this stage. Moreover, the diabetes and nutrition study group of the Spanish Diabetes Association, in a 7-year prospective, population-based, multicenter study showed that normoalbuminuria and nephropathy regression in well-controlled diabetic patients with long-term diabetes (type 1 and 2) are associated with enhanced PUFAs and less saturated fatty acid consumption [119].

3. Conclusions

Considerable progress has been made in understanding how n-3 PUFAs (from fish oil) affect cell function. In addition to their use as a fuel and structural component of cells, dietary n-3 fatty acids appear to play an important protecting role against the adverse symptoms of the "Plurimetabolic syndrome," preventing or ameliorating coronary heart disease and stroke. The effects of dietary PUFAs include, among others, (a) the alteration of the fatty acid composition of membrane phospholipids that modify membrane-mediated processes such as insulin transduction signals, activity of lipases and synthesis of eicosanoids, and (b) the regulation of nuclear events that govern specific gene transcription (e.g., genes involved in lipid and glucose metabolism and adipogenesis). However, some effects of n-3 PUFAs on physiological processes in both humans and experimental animals still remain unclear and need further research. For instance, a better comprehension of the nature of the intracellular signal responsible for regulating the various affected transcription factors will undoubtedly contribute to understanding how these singular lipids impact upon human health and disease.

Acknowledgments

The authors acknowledge the financial support received from UNL, CONICET, ANPCyT, A.J. Roemmers Foundation of Biochemistry Research and R. Carrillo-A. Oñativia Fellowship, Argentina. They are also grateful to Professors R. Gutman and J.C. Basabe and to the numerous graduate students and postdoctoral fellows who have participated in this research project. Thanks are also given to Professors R.R. Brenner and J.J. Gagliardino for their specialized contribution.

References

[1] Din JN, Newby DE, Flapan AD. Omega 3 fatty acids and cardiovascular disease—fishing for a natural treatment. BMJ 2004;328:30–5.
[2] Harris WS, Hustvedt BE, Hagen E, Green MH, Lu G, Drevon CA. N-3 fatty acids and chylomicron metabolism in the rat. J Lipid Res 1997;38:503–15.
[3] Mori TA, Burke V, Puddey IB, Watts GF, O'Neal DN, Best JD, et al. Purified eicosapentaenoic and docosahexaenoic acids have differential effects on serum lipids and lipoproteins, LDL particle size, glucose, and insulin in mildly hyperlipidemic men. Am J Clin Nutr 2000;71:1085–94.
[4] Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation 2002; 106:2747–57.
[5] Connor WE. Importance of n-3 fatty acids in health and disease. Am J Clin Nutr 2000;71(Suppl):171S–5S.
[6] Hwang D. Fatty acids and immune responses — a new perspective in searching for clues to mechanism. Annu Rev Nutr 2000;20:431–56.
[7] Jump DB. The biochemistry of n-3 polyunsaturated fatty acids. J Biol Chem 2002;277:8755–8.
[8] Salem Jr N, Litman B, Kim HY, Gawrisch K. Mechanisms of action of docosahexaenoic acid in the nervous system. Lipids 2001;36: 945–59.
[9] Simopoulos AP. Essential fatty acids in health and chronic disease. Am J Clin Nutr 1999;70(Suppl):560S–9S.
[10] Geleijnse JM, Giltay EJ, Grobbee DE, Donders ART, Kok FJ. Blood pressure response to fish oil supplementation: metaregression analysis of randomized trials. J Hypertension 2002;20:1493–9.
[11] Storlien LH, Hulbert AJ, Else PL. Polyunsaturated fatty acids, membrane function and metabolic diseases such as diabetes and obesity. Curr Opin Clin Nutr Metab Care 1998;1:559–63.
[12] Kim HJ, Takahashi M, Ezaki O. Fish oil feeding decreases mature sterol regulatory element-binding protein 1 (SREBP-1) by down-regulation of SREBP-1c mRNA in mouse liver. J Biol Chem 1999; 274:25892–8.
[13] Clarke SD. Polyunsaturated fatty acid regulation of gene transcription: a molecular mechanism to improve the metabolic syndrome. J Nutr 2001;131:1129–32.
[14] Takahashi M, Tsuboyama-Kasaoka N, Nakatani T, Ishii M, Tsutsumi S, Aburatani H, et al. Fish oil feeding alters liver gene expressions to defend against PPARα activation and ROS production. Am J Physiol 2002;282:G338–48.
[15] Reaven GM, Risser TR, Chen YDI, Reaven EP. Characterization of a model of dietary induced hypertriglyceridemia in young, non-obese rats. J Lipid Res 1979;20:371–8.
[16] Reaven GM. Diabetic hypertriglyceridemia in the rat: animal models simulating the clinical syndromes of impaired glucose tolerance, noninsulin-dependent diabetes and insulin-dependent diabetes. In: Shafrir E, Renold AS, editors. Lessons from animal diabetes. London (UK): Libby; 1984. p. 531–6.
[17] Vrana A, Kazdova L, Dobesova Z, Kunes J, Kren V, Bila V, et al. Triglyceridemia, glucoregulation, and blood pressure in various rat strains. Effects of dietary carbohydrates. In: Klimes I, Howard BV, Storlien LH, Sebokova E, editors. Dietary lipids and insulin action. Second International Smolenice Insulin Symposium, vol 683. Ann N Y Acad Sci 1993. p. 57–68.
[18] Pagliassotti MJ, Shahrokhi KA, Moscarello M. Involvement of liver and skeletal muscle in sucrose-induced insulin resistance: dose-response studies. Am J Physiol 1994;266:R1637–44.
[19] Pagliassotti MJ, Prach PA, Koppenhafer TA, Pan DA. Changes in insulin action, triglycerides, and lipid composition during sucrose feeding in rats. Am J Physiol 1996;271:R1319–26.
[20] Lombardo YB, Chicco A, Mocchiutti N, Rodi MA, Nusimovich B, Gutman R. Effect of sucrose diet on insulin secretion in vivo and in vitro and on triglycerides storage and mobilisation of the heart of rats. Horm Metab Res 1983;15:69–76.
[21] Chicco A, Gutman R, Lombardo YB. Biochemical abnormalities in the heart of rats fed a sucrose-rich diet: is the low activity of the pyruvate dehydrogenase complex a result of increased fatty acid oxidation? Metabolism 1991;40:15–21.
[22] Bernal C, Gutman R, Lombardo YB. The duration of feeding on a sucrose-rich diet determines variable in vitro effects of insulin and fructose in rat liver triglyceride metabolism. J Nutr Biochem 1995;6:422–30.
[23] Shafrir E. Effect of sucrose and fructose on carbohydrate and lipid metabolism and the resulting consequences. In: Beitner R, editor. Regulation of carbohydrate metabolism. Boca Raton (Fla): CRC; 1985. p. 95–140.
[24] Rizkalla SW, Luo J, Guilhem I, Boillot J, Bruzzo F, Chevalier A, et al. Comparative effects of 6 weeks fructose, dextrose and starch feeding on fat-cell lipolysis in normal rats: effects of isoproterenol, theophylline and insulin. Mol Cell Biochem 1992;109: 127–32.
[25] Storlien LH, Kraegen EW, Jenkins AB, Chisholm DJ. Effects of sucrose vs starch diets on in vivo insulin action, thermogenesis, and obesity in rats. Am J Clin Nutr 1988;47:420–7.
[26] Bezerra RMN, Ueno M, Silva MS, Tavares DQ, Carvalho CRO, Saad MJA. A high fructose diet affects the early steps of insulin action in muscle and liver of rats. J Nutr 2000;130:1531–5.
[27] Luo J, Rizkalla SW, Lerer-Metzger M, Boillot J, Ardeleanu A, Bruzzo F, et al. A fructose-rich diet decreases insulin-stimulated glucose incorporation into lipids but not glucose transport in adipocytes of normal and diabetic rats. J Nutr 1995;125:164–71.
[28] Soria A, D'Alessandro ME, Lombardo YB. Duration of feeding on a sucrose-rich diet determines metabolic and morphological changes in rat adipocyte. J Appl Physiol 2001;91:2109–16.
[29] Cheal KL, Abbasi F, Lamendola C, McLaughlin T, Reaven GM, Ford ES. Relationship to insulin resistance of the adult treatment panel III diagnostic criteria for identification of the metabolic syndrome. Diabetes 2004;53:1195–200.
[30] Klimes I, Sebokova E, Vrana A, Kazdova L. Raised dietary intake of n-3 polyunsaturated fatty acids in high sucrose-induced insulin resistance. Animal studies. In: Klimes I, Howard BV, Storlien LH, Sebokova E, editors. Dietary lipids and insulin action. Second International Smolenice Insulin Symposium, vol 683. Ann N Y Acad Sci 1993. p. 69–81.
[31] Podolin DA, Gayles EC, Wei Y, Thresher JS, Pagliassotti MJ. Menhaden oil prevents but does not reverse sucrose-induced insulin resistance in rats. Am J Physiol 1998;274:R840–8.
[32] Peyron-Caso E, Fluteau-Nadler S, Kabir M, Guerre-Millo M, Quignard-Boulange A, Slama G, et al. Regulation of glucose transport and transporter 4 (Glut-4) in muscle and adipocytes of sucrose-fed rats: effects of n-3 poly- and monounsaturated fatty acids. Horm Metab Res 2002;34:362–6.
[33] Herzberg GR, Rogerson M. Hepatic fatty acid synthesis and triglyceride secretion in rats fed fructose- or glucose-based diets containing corn oil, tallow or marine oil. J Nutr 1988;118:1061–7.

[34] Clarke SD, Jump DB. Dietary polyunsaturated fatty acid regulation of gene transcription. Annu Rev Nutr 1994;14:83–98.
[35] Pegorier JP. Regulation of gene expression by fatty acids. Curr Opin Clin Nutr Metab Care 1998;1:329–34.
[36] Jump DB, Clarke SD. Regulation of gene expression by dietary fat. Annu Rev Nutr 1999;19:63–90.
[37] Lang CA, Davis RG. Fish oil fatty acids impair VLDL assembly and/or secretion by cultured rat hepatocytes. J Lipid Res 1990;31: 2079–86.
[38] Baker PW, Gibbons GF. Effect of dietary fish oil on the sensitivity o hepatic lipid metabolism to regulation by insulin. J Lipid Res 2000; 41:719–26.
[39] Halvorsen B, Rustan AC, Madsen L, Reseland J, Berge RK, Sletnes P, et al. Effects of long-chain monounsaturated and n-3 fatty acids on fatty acid oxidation and lipid composition in rats. Ann Nutr Metab 2001;45:30–7.
[40] Neschen S, Moore I, Regittnig W, Yu CL, Wang Y, Pypaert M, et al. Contrasting effects of fish oil and safflower oil on hepatic peroxisomal and tissue lipid content. Am J Physiol 2002;282:E395–E401.
[41] Berge RK, Madsen L, Vaagenes H, Tronstad KJ, Gottlicher M, Rustan AC. In contrast with docosahexaenoic acid, eicosapentaenoic acid and hypolipidaemic derivatives decrease hepatic synthesis and secretion of triacylglycerol by decreased diacylglycerol acyltransferase activity and stimulation of fatty acid oxidation. Biochem J 1999;343:191–7.
[42] Pan DA, Mater MK, Thelen AP, Peters JM, Gonzalez FJ, Jump DB. Evidence against the peroxisome proliferator-activated receptor α(PPARα) as the mediator for polyunsaturated fatty acid suppression of hepatic L-pyruvate kinase gene transcription. J Lipid Res 2000;41:742–51.
[43] Ren B, Thelen AP, Peters JM, Gonzalez FJ, Jump DB. Polyunsaturated fatty acid suppression of hepatic fatty acid synthase and S14 gene expression does not require peroxisome proliferator-activated receptor α. J Biol Chem 1997;272:26827–32.
[44] Peyron-Caso E, Quignard-Boulange A, Laromiguiere M, Feing-Knwong-Chan S, Veronese A, Ardouin B, et al. Dietary fish oil increases lipid mobilization but does not decrease lipid storage-related enzymes activities in adipose tissue of insulin-resistant, sucrose-fed rats. J Nutr 2003;133:2239–43.
[45] Peyron-Caso E, Taverna N, Guerre-Millo M, Veronese A, Pacher N, Slama G, et al. Dietary (n-3) polyunsaturated fatty acids up-regulate plasma leptin in insulin-resistant rats. J Nutr 2002;132:2235–40.
[46] Reseland JE, Haugen F, Hollung K, Solvoll K, Halvorsen B, Brude IR, et al. Reduction of leptin gene expression by dietary polyunsaturated fatty acids. J Lipid Res 2001;42:743–50.
[47] Thorburn AW, Storlien LH, Jenkins AB, Khouri S, Kraegen EW. Fructose-induced in vivo insulin resistance and elevated plasma triglycerides levels in rats. Am J Clin Nutr 1989;49:1155–63.
[48] Clamp AG, Ladha S, Clark DC, Grimble RF, Lund EK. The influence of dietary lipids on the composition and membrane fluidity of rat hepatocyte plasma membrane. Lipids 1997;32:179–84.
[49] Storlien LH, Pan DA, Kriketos AD, O'Connor J, Caterson ID, Cooney GJ, et al. Skeletal muscle membrane lipids and insulin resistance. Lipids 1996;31:S261–5.
[50] Luo J, Rizkalla SW, Boillot J, Alamowitch C, Chaib H, Bruzzo F, et al. Dietary (n-3) polyunsaturated fatty acids improve adipocyte insulin action and glucose metabolism in insulin-resistant rats: relation to membrane fatty acids. J Nutr 1996;126:1951–8.
[51] Rizkalla SW, Alamowitch C, Luo J, Bruzzo F, Boillot A, Chevalier A, et al. Effect of dietary fish oil on insulin action in fat cells of control and non-insulin-dependent rats. In: Klimes I, Howard BV, Storlien LH, Sebokova E, editors. Dietary lipids and insulin action. Second International Smolenice Insulin Symposium, vol 683. Ann N Y Acad Sci 1993. p. 213–7.
[52] Sebokova E, Klimes I, Gasperikova D, Bohov P, Langer P, Lavau M, et al. Regulation of gene expression for lipogenic enzymes in liver and adipose tissue of hereditary hypertriglyceridemic, insulin-resistant rats: effect of dietary sucrose and marine fish oil. Biochim Biophys Acta 1996;1303:56–62.
[53] Mori Y, Murakawa Y, Katoh S, Hata S, Yokoyama J, Tajima N, et al. Influence of highly purified eicosapentaenoic acid ethyl ester on insulin resistance in the Otsuka Long–Evans Tokushima fatty rat, a model of spontaneous non-insulin-dependent diabetes mellitus. Metabolism 1997;46:1458–64.
[54] Storlien LH, Pan DA, Kriketos AD, Baur LA. High fat diet-induced insulin resistance. Lesson and implications from animal studies. In: Klimes I, Howard BV, Storlien LH, Sebokova E, editors. Dietary lipids and insulin action. Second International Smolenice Insulin Symposium, vol 683. Ann N Y Acad Sci 1993. p. 82–90.
[55] Storlien LH, Jenkins AB, Chisholm DJ, Pascoe WS, Khouri S, Kraegen EW. Influence of dietary fat composition on development of insulin resistance in rats. Relationship to muscle triglyceride and w-3 fatty acids in muscle phospholipid. Diabetes 1991;40:280–9.
[56] Oakes ND, Cooney GJ, Camilleri S, Chisholm DJ, Kraegen EW. Mechanisms of liver and muscle insulin resistance induced by chronic high-fat feeding. Diabetes 1997;46:1768–74.
[57] Oakes ND, Bell KS, Furler SM, Camilleri S, Saha AK, Ruderman NB, et al. Diet-induced muscle insulin resistance in rats is ameliorated by acute dietary lipid withdrawal or a single bout of exercise: parallel relationship between insulin stimulation of glucose uptake and suppression of long-chain fatty acyl-CoA. Diabetes 1997;46:2022–8.
[58] Kraegen EW, Cooney GJ. The role of free fatty acids in muscle insulin resistance. Diabetes Annu 1999;12:141–59.
[59] Schmitz-Peiffer C, Browne CL, Oakes ND, Watkinson A, Chisholm DJ, Kraegen EW, et al. Alteration in the expression and cellular localization of protein kinase C isozymes ϵ and θ are associated with insulin resistance in skeletal muscle of the high-fat-fed rat. Diabetes 1997;46:169–78.
[60] Bell KS, Schmitz-Peiffer C, Lim-Fraser M, Biden TJ, Cooney GJ, Kraegen EW. Acute reversal of lipid-induced muscle insulin resistance is associated with rapid alteration in PKCθ localization. Am J Physiol 2000;279:E1196–201.
[61] Taouis M, Dagou C, Ster C, Durand G, Pinault M, Delarue J. N-3 polyunsaturated fatty acids prevent the defect of insulin receptor signaling in muscle. Am J Physiol 2002;282:E664–71.
[62] Storlien LH, Kraegen EW, Chisholm DJ, Ford GL, Bruce DG, Pascoe WS. Fish oil prevents insulin resistance induced by high-fat feeding in rats. Sciences 1987;237:885–8.
[63] Borkman M, Storlien LH, Pan DA, Jenkins AB, Chisholm DJ, Campbell LV. The relation between insulin sensitivity and the fatty-acid composition of skeletal-muscle phospholipids. N Engl J Med 1993;328:238–44.
[64] Storlien LH, Baur LA, Kriketos AD, Pan DA, Cooney GJ, Jenkins AB, et al. Dietary fats and insulin action. Diabetologia 1996;39: 621–31.
[65] Wilkes JJ, Bonen A, Bell RC. A modified high-fat diet induces insulin resistance in rat skeletal muscle but not adipocytes. Am J Physiol 1998;275:E679–86.
[66] Kim JY, Nolte LA, Hansen PA, Han DH, Ferguson K, Thompson PA, et al. High-fat diet-induced muscle insulin resistance: relationship to visceral fat mass. Am J Physiol 2000;279:R2057–65.
[67] Okuno M, Kajiwara K, Imai S, Kobayashi T, Honma N, Maki T, et al. Perilla oil prevents the excessive growth of visceral adipose tissue in rats by down-regulating adipocyte differentiation. J Nutr 1997;127: 1752–7.
[68] Hainault I, Carlotti M, Hajduch E, Guichard C, Lavau M. Fish oil in a high lard diet prevents obesity, hyperlipemia and adipocyte insulin resistance in rats. In: Klimes I, Howard BV, Storlien LH, Sebokova E editors. Dietary lipids and insulin action. Second International Smolenice Insulin Symposium, vol 683. Ann N Y Acad Sci 1993. p. 98–101.
[69] Ohinata H, Saha SK, Ohno T, Hata N, Misawa Y, Kuroshima A. Effect of dietary docosahexaenoic acid on in vitro thermogenesis and

fatty acid compositions of brown adipose tissue. Jpn J Physiol 1998;48:189–96.
[70] Kawada T, Kayahashi S, Hida Y, Koga K, Nadachi Y, Fushiki T. Fish (Bonito) oil supplementation enhances the expression of uncoupling protein in brown adipose tissue of rat. J Agric Food Chem 1998;46:1225–7.
[71] Takahashi Y, Takashi I. Dietary n-3 fatty acids affect m-RNA level of brown adipose tissue uncoupling protein 1, and white adipose tissue leptin and glucose transporter 4 in the rat. Br J Nutr 2000;84:175–84.
[72] Benhizia F, Hainault I, Serougne C, Lagrange D, Hajduch E, Guichard C, et al. Effects of a fish oil-lard diet on rat plasma lipoproteins, liver FAS, and lipolytic enzymes. Am J Physiol 1994;267:E975–82.
[73] Raclot T, Groscolas R, Langin D, Ferre P. Site-specific regulation of gene expression by n-3 polyunsaturated fatty acids in rat white adipose tissues. J Lipid Res 1997;38:1963–72.
[74] Masuzaki H, Ogawa Y, Hosoda K, Kawada T, Fushiki T, Nakao K. Augmented expression of the obese gene in the adipose tissue from rats fed high-fat diet. Biochem Biophys Res Commun 1995;216: 355–8.
[75] Rousseau V, Becker DJ, Ongemba LN, Rahier J, Henquin JC, Brichard SM. Developmental and nutritional changes of *ob* and PPAR γ2 gene expression in rat white adipose tissue. Biochem J 1997;321:451–6.
[76] Pedersen O, Khan CR, Flier JS, Khan BB. High fat feeding causes insulin resistance and a marked decrease in the expression of glucose transporters (Glut 4) in fat cells of rats. Endocrinology 1991;129: 771–7.
[77] Sevilla L, Guma A, Enrique-Tarancon G, Mora S, Muñoz P, Palacin M, et al. Chronic high-fat feeding and middle-aging reduce in an additive fashion Glut 4 expression in skeletal muscle and adipose tissue. Biochem Biophys Res Commun 1997;235:89–93.
[78] Ezaki O, Tsuji E, Momomura K, Kasuga M, Itakura H. Effects of fish and safflower oil feeding on subcellular glucose transporter distributions in rat adipocytes. Am J Physiol 1992;263:E94–E101.
[79] Willson TM, Lambert MH, Kliewer SA. Peroxisome proliferator-activated receptor γ and metabolic disease. Annu Rev Biochem 2001;70:341–67.
[80] Gutman RA, Basilico MZ, Bernal C, Chicco A, Lombardo YB. Long-term hypertriglyceridemia and glucose intolerance in rats fed chronically and isocaloric sucrose-rich diet. Metabolism 1987;36: 1013–20.
[81] Chicco A, Soria A, Fainstein-Day P, Gutman R, Lombardo YB. Multiphasic metabolic changes in the heart of rats fed a sucrose-rich diet. Horm Metab Res 1994;26:397–403.
[82] Montes M, Chicco A, Lombardo YB. The effect of insulin on the uptake and metabolic fate of glucose in isolated perfused hearts of dyslipemic rats. J Nutr Biochem 2000;11:30–7.
[83] Chicco A, D'Alessandro ME, Karabatas L, Pastorale C, Basabe JC, Lombardo YB. Muscle lipid metabolism and insulin secretion are altered in insulin-resistant rats fed a high sucrose diet. J Nutr 2003; 133:127–33.
[84] Brenner RR, Rimoldi OJ, Lombardo YB, Gonzalez MS, Bernasconi M, Chicco A, et al. Desaturase activities in rat model of insulin resistance induced by a sucrose-rich diet. Lipids 2003;38:733–42.
[85] Lombardo YB, Rossi A, LaCorte JM, Chicco A, Rouault C, Rizkalla SW. Functional and morphological changes in adipose tissue of insulin resistant rats fed a sucrose-rich diet. Effects of dietary fish oil. 22nd International Symposium on Diabetes and Nutrition, Sastaholm, Sweeden. 2004. p. 3.
[86] Lombardo YB, Drago S, Chicco A, Fainstein-Day P, Gutman R, Gagliardino JJ, et al. Long-term administration of a sucrose-rich diet to normal rats: relationship between metabolic and hormonal profiles and morphological changes in the endocrine pancreas. Metabolism 1996;45:1527–32.
[87] Lombardo YB, Chicco A, D'Alessandro ME, Martinelli M, Soria A, Gutman R. Dietary fish oil normalize dyslipidemia and glucose intolerance with unchanged insulin levels in rats fed a high sucrose diet. Biochem Biophys Acta 1996;1299:175–82.
[88] D'Alessandro ME, Chicco A, Karabatas L, Lombardo YB. Role of skeletal muscle on impaired insulin sensitivity in rats fed a sucrose-rich diet: effect of moderate levels of dietary fish oil. J Nutr Biochem 2000;11:273–80.
[89] Randle PJ. Regulatory interactions between lipids and carbohydrates: the glucose fatty acid cycle after 35 years. Diabetes Metab Rev 1998;14:263–83.
[90] Pighin D, Karabatas L, Rossi A, Chicco A, Basabe JC, Lombardo YB. Fish oil affects pancreatic fat storage, pyruvate dehydrogenase complex activity and insulin secretion in rats fed a sucrose-rich diet. J Nutr 2003;133:4095–101.
[91] Mc Garry JD, Dobbins RL. Fatty acids, lipotoxicity and insulin secretion. Diabetologia 1999;42:128–38.
[92] Unger RH. Lipotoxicity in the pathogenesis of obesity-dependent NIDDM: genetic and clinical implications. Diabetes 1995;44: 863–70.
[93] Rossetti L, Guiaccari A, DeFronzo RA. Glucose toxicity. Diabetes Care 1990;13:610–29.
[94] Sturis L, Scheem AJ, Leproult R, Polonsky KS, Van Cauter E. 24-hour glucose profiles during continuous or oscillatory insulin infusion. Demonstration of the functional significance of ultradian insulin oscillations. J Clin Invest 1995;95:1464–71.
[95] Briaud I, Harmon JS, Kelpe CL, Segu VB, Poitout V. Lipotoxicity of the pancreatic β-cell is associated with glucose-dependent esterification of fatty acids into neutral lipids. Diabetes 2001;50:315–21.
[96] Lewis GF, Carpentier A, Khospow A, Giacca A. Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. Endocr Rev 2002;23:201–29.
[97] Unger RH, Orci L. Diseases of liporegulation: new perspective on obesity and related disorders. FASEB J 2001;15:312–21.
[98] Man ZW, Zhu N, Noma Y, Toide K, Sato T, Asahi Y, et al. Impaired β-cell function and deposition of fat droplets in the pancreas as a consequence of hypertriglyceridemia in OLETF rat, a model of spontaneous NIDDM. Diabetes 1997;46:1718–24.
[99] Soria A, Chicco A, D'Alessandro ME, Rossi A, Lombardo YB. Dietary fish oil reverse epididymal tissue adiposity, cell hypertrophy and insulin resistance in dyslipemic sucrose fed rat model. J Nutr Biochem 2002;13:209–18.
[100] Havel PJ. Update on adipocyte hormones. Regulation of energy balance and carbohydrate/lipid metabolism. Diabetes 2004;53: S143–51.
[101] Fontbonne A, Eschwege E, Cambien F, Richard JL, Ducimetiere P, Thibult N, et al. Hypertriglyceridemia as a risk factor of coronary heart disease mortality in subjects with impaired glucose tolerance or diabetes. Results from the 11-year follow-up of the Paris Prospective Study. Diabetologia 1989;32:300–4.
[102] Hokanson JE, Austin MA. Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. J Cardiovasc Risk 1996;3:213–9.
[103] Hansen JB, Grimsgaard S, Nilsen H, Nordoy A, Bonaa KH. Effects of highly purified eicosapentaenoic acid and docosahexaenoic acid on fatty acid absorption, incorporation into serum phospholipids and postprandial triglyceridemia. Lipids 1998;33:131–8.
[104] Couet C, Dalarue J, Ritz P, Antoine JM, Lamisse F. Effect of dietary fish oil on body mass and basal fat oxidation in healthy adults. Int J Obesity 1997;21:637–43.
[105] Zhengling L, Lamon-Fava S, Otvos J, Lichtenstein AH, Velez-Carrasco W, McNamara JR, et al. Fish consumption shifts lipoprotein subfractions to a less atherogenic pattern in humans. J Nutr 2004;134:1724–8.
[106] Tinker LF, Parks EJ, Behr SR, Schneeman BO, Davis PA. (n-3) fatty acids supplementation in moderately hypertriglyceridemic adults changes postprandial lipid and apolipoprotein B responses to a standardized test meal. J Nutr 1999;129:1126–34.

[107] Garcia-Pelayo MC, Garcia-Peregrin E, Martinez-Cayuela M. Differential translational effects of myristic acid and eicosapentaenoic acid on 3-hydroxy-3-methylglutaryl-CoA reductase from Reuber H35 hepatoma cells. Exp Biol Med 2004;229:781–6.
[108] Nestel PJ. Fish oil and cardiovascular disease: lipids and arterial function. Am J Clin Nutr 2000;71:228–31.
[109] Harper CR, Jacobson TA. The fats of life: the role of omega-3-fatty acids in the prevention of coronary heart disease. Arch Intern Med 2001;161:2185–92.
[110] Bao DQ, Mori TA, Burke V, Puddey IB, Beilin LJ. Effects of dietary fish and weigh reduction on ambulatory blood pressure in overweight hypertensives. Hypertension 1998;32:710–7.
[111] Feskens E, Bowles CH, Kromhout D. Inverse association between fish intake and risk of glucose intolerance in normoglycemic men and women. Diabetes Care 1991;14:935–41.
[112] Stene LC, Joner G, Norwegian Childhood Diabetes Study Group. Use of cod liver oil during the first year of life is associated with lower risk of childhood-onset type 1 diabetes: a large, population-based, case-control study. Am J Clin Nutr 2003;78:1128–34.
[113] Glauber H, Wallace P, Griver K, Brechtel G. Adverse metabolic effect of omega-3 fatty acids in non-insulin-dependent diabetes mellitus. Ann Intern Med 1988;108:663–8.
[114] Friday KE, Childs MT, Tsunehara CH, Fujimoto WY, Bierman EL, Ensinck JW. Elevated plasma glucose and lowered triglyceride levels from omega 3 fatty acid supplementation in type II diabetes. Diabetes Care 1989;12:276–81.
[115] Rivellese AA, Maffettone A, Iovine C, DiMarino L, Annuzzi G, Mancini M, et al. Long-term effects of fish oil on insulin resistance and plasma lipoproteins in NIDDM patients with hypertriglyceridemia. Diabetes Care 1996;19:1207–13.
[116] Popp-Snijders C, Schouten JA, Heine RJ, van der Meer J, van der Veen EA. Dietary supplementation of w-3 polyunsaturated fatty acids improves insulin sensitivity in non-insulin-dependent diabetes. Diabetes Res 1987;4:141–7.
[117] Friedberg CE, Janssen MJEM, Heine RJ, Grobbee DE. Fish oil and glycemic control in diabetes. A meta-analysis. Diabetes Care 1998; 21:494–500.
[118] Skurnick-Minot G, Laromiguiere M, Oppert JM, Quignard-BoulanGE A, Boillot J, Rigoir A, et al. Whole-body fat mass and insulin sensitivity in type 2 diabetic women: effect of n-3 polyunsaturated fatty acids. 64th ADA Meeting, Orlando, June 4th–8th, 2004, Diabetes 2004;53 Suppl 2, A44 (0159).
[119] The Diabetes and Nutrition Study Group of the Spanish Diabetes Association (GSEDNU). Polyunsaturated fatty acid consumption may play a role in the onset and regression of microalbuminuria in well-controlled Type 1 and Type 2 diabetic people. A 7-year prospective, population-based, observational multicenter study. Diabetes Care 2004;27:1454–7.