Reprod. Nutr. Dev. 44 (2004) 289–299

DOI: 10.1051/rnd:2004033

Review article

n-3 long chain polyunsaturated fatty acids: a nutritional tool to prevent insulin resistance associated to type 2 diabetes and obesity?

Jacques DELARUE[a,b]*, Christelle LEFOLL[a], Charlotte CORPOREAU[a], Danièle LUCAS[a]

[a] EA-948 "Oxylipides", Faculté de Médecine, 29200 Brest, France
[b] Laboratoire Régional de Nutrition Humaine, CHU Cavale Blanche, 29200 Brest, France

Abstract – n-3 long chain polyunsaturated fatty acids (n-3 LC-PUFA), mainly eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3), are present in mammal tissues both from endogenous synthesis from desaturation and elongation of 18:3 n-3 and/or from dietary origin (marine products and fish oils). In rodents in vivo, n-3 LC-PUFA have a protective effect against high fat diet induced insulin resistance. Such an effect is explained at the molecular level by the prevention of many alterations of insulin signaling induced by a high fat diet. Indeed, the protective effect of n-3 LC-PUFA results from the following: (a) the prevention of the decrease of phosphatidyl inositol 3' kinase (PI3 kinase) activity and of the depletion of the glucose transporter protein GLUT4 in the muscle; (b) the prevention of the decreased expression of GLUT4 in adipose tissue. In addition, n-3 LC-PUFA inhibit both the activity and expression of liver glucose-6-phosphatase which could explain the protective effect with respect to the excessive hepatic glucose output induced by a high fat diet. n-3 LC-PUFA also decrease muscle intramyofibrillar triglycerides and liver steatosis. This last effect results on the one hand, from a decreased expression of lipogenesis enzymes and of delta 9 desaturase (via a depleting effect on sterol response element binding protein 1c (SREBP-1c). On the other hand, n-3 LC-PUFA stimulate fatty acid oxidation in the liver (via the activation of peroxisome proliferator activated receptor α (PPAR-α)). In patients with type 2 diabetes, fish oil dietary supplementation fails to reverse insulin resistance for unclear reasons, but systematically decreases plasma triglycerides. Conversely, in healthy humans, fish oil has many physiological effects. Indeed, fish oil reduces insulin response to oral glucose without altering the glycaemic response, abolishes extraggression at times of mental stress, decreases the activation of sympathetic activity during mental stress and also decreases plasma triglycerides. These effects are encouraging in the perspective of prevention of insulin resistance but further clinical and basic studies must be designed to confirm and complete our knowledge in this field.

eicosapentaenoic acid (EPA) / docosahexaenoic acid (DHA) / peroxisome proliferator-activated receptors (PPAR) / non alcoholic steatohepatitis / glucose metabolism / insulin resistance

Abbreviations
EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; PPAR: peroxisome proliferator-activated receptors; NASH: non alcoholic steatohepatitis; n-3 LC-PUFA: n-3 long chain polyunsaturated fatty acids. SREBP-1c: sterol response element binding protein 1c; IRS-1: insulin receptor substrate-1; PI3 kinase: phosphatidyl inositol 3' kinase; NEFA: non esterified fatty acids; LCACoA: long chain acyl CoA; PKC: protein kinase C; HNF4: hepatic nuclear factor 4; NAFLD: nonalcoholic fatty liver disease; G6Pase: glucose 6 phosphatase.

* Corresponding author: jacques.delarue@univ-brest.fr

1. INTRODUCTION

Insulin resistance that is a reduced efficacy of insulin in vivo to inhibit hepatic glucose production and to stimulate glucose utilization in skeletal muscle and adipose tissue, is a common characteristic of type 2 diabetes, obesity and metabolic syndrome. At the cellular and molecular levels, many defects of the insulin signaling pathway have been demonstrated. These defects are in the case of obesity essentially the consequence of an excess dietary intake and in the case of type 2 diabetes primitively determined by genetic defects leading to metabolic alterations which are aggravated by environmental factors, including the diet and inactivity. Genetic defects predisposing to type 2 diabetes are under intensive investigation and remain currently essentially unknown. Conversely, the role of environmental factors is better known. Epidemiologic cross sectional and longitudinal studies have demonstrated the deleterious effect of inactivity on insulin sensitivity and the beneficial effect of physical activity to prevent or to reverse insulin resistance. Dietary factors, mainly fat and some types of carbohydrates are strongly suspected to induce or to aggravate insulin resistance in animals as well as in humans. If we consider exclusively fat as a contributor to insulin resistance, it appears that an excess of fat intake and especially an excess of saturated fat intake predisposes to insulin resistance. Animal studies have demonstrated that the quality of fat is as important as its quantity to determine or not insulin resistance. Clearly, n-3 long chain fatty acids contained in fish oils have a protective effect against insulin resistance in rodents fed a high fat diet. Many basic cellular and molecular mechanisms have been discovered which can explain this protective effect. Unfortunately, fish oil dietary supplementation does not reverse insulin resistance in patients with type 2 diabetes. The reasons underlying this discrepancy between animal studies and type 2 diabetics remain obscure even though some hypotheses can be proposed. In vivo studies in healthy humans demonstrate or strongly suggest that n-3 LC-PUFA modulate intermediary metabolism in a way that could help to prevent insulin resistance.

In the present review, we will focus on the following: (a) on the main cellular defects of insulin signaling described during type 2 diabetes and obesity which could be a target for prevention by n-3 LC-PUFA; (b) on the available data suggesting that they could modulate or not intermediary metabolism in vivo in rodents and humans. The specific effects of n-3 LC-PUFA on adipose tissue metabolism and differentiation will not be treated in the present review.

2. MOLECULAR DEFECTS IN INSULIN-DEPENDENT TISSUES FROM PATIENTS WITH TYPE 2 DIABETES

Type 2 diabetes results from the association of a defect in insulin secretion from ß-cells and insulin resistance in the muscle, adipose tissue and the liver. Muscle is the main peripheral tissue concerned by insulin resistance during type 2 diabetes. Indeed, glucose uptaken by the muscle during hyperinsulinaemia accounts for about 75% of whole body glucose utilization, while glucose uptaken by adipose tissue accounts for 1–2% only. Clearly, insulin signaling is altered in the muscle from obese patients with type 2 diabetes. This alteration is characterized by an abolition, during hyperinsulinaemia, of the stimulation of the phosphorylation of insulin receptor substrate-1 (IRS-1) and of the stimulation of phosphatidyl inositol 3' (PI3 kinase) activity [1]. An impaired insulin action on PI3 kinase activity has also been reported in obese insulinoresistant patients with normal glucose tolerance [2]. These defects are not associated with a reduced expression of the insulin receptor, IRS-1, or the p85 subunit of PI3-kinase [3]. The decrease in PI3 kinase activity is in accordance with a decreased glucose transport since PI3 kinase activity stimulates

glucose transporter GLUT4 translocation. The GLUT4 protein is decreased in slow fibers from the muscle of obese and type 2 diabetics [4]. In addition, in type 2 diabetes, muscle GLUT4 translocation during hyperinsulinaemia has been demonstrated to be reduced by 90% [5]. Alterations of insulin signaling only alter metabolic pathways; signal transduction along the mitogen-activated protein (MAP) kinase pathway is normal [2, 3].

3. BODY FAT AND INSULIN SENSITIVITY

Cross-sectionnal [6] and prospective [7] studies have shown an association between obesity and type 2 diabetes. This association is, at least for a part, related to insulin resistance. During obesity, insulin resistance occurs in the liver, muscle and adipose tissue but it is thought that the muscle is the predominant tissue responsible for insulin resistance [8]. Body fatness and visceral adiposity are both independently related to insulin resistance. Visceral obesity is a stronger determinant of the risk of developing insulin resistance than body or subcutaneous increased fat mass. Development of insulin resistance in patients with visceral obesity is attributed to the greater lipolytic activity of omental adipocytes as compared to subcutaneous adipocytes. It has been proposed that the increased delivery of non esterified fatty acids (NEFA) into the portal vein, and then their increased appearance in systemic circulation is, according to the Randle cycle, responsible for the development of insulin resistance. However, insulin resistance is not only related to visceral fat mass but is also independently related to subcutaneous fat mass. This observation strongly suggests that other mechanisms than the portal NEFA delivery are implicated in insulin resistance during visceral obesity. In addition, the physiological responsibility of the Randle cycle in insulin-resistance development has been challenged. For these reasons, new concepts have recently been developed to try to explain physiological and basic mechanisms of insulin-resistance during obesity. The main new concept is that of "ectopic fat storage syndrome". Moreover, adipose tissue known to be an endocrine organ [9], is currently considered as the primary tissue responsible for insulin-resistance.

3.1. Ectopic fat syndrome and insulin resistance

3.1.1. Muscle triglycerides

Strong arguments exist which relate muscle insulin resistance to an excess of triglyceride content in myofibrillar cells [10]. This excess of skeletal muscle triglycerides has been observed in obese and in type 2 diabetics [11, 12]. It results from a metabolic inflexibility of lipid oxidation. In leg muscles, the rate of NEFA uptake has been demonstrated to be similar in obese and lean subjects; Conversely, in the same tissue, fat oxidation was shown to be decreased in the fasting state and increased by insulin stimulation in obese subjects suggesting an operative Randle cycle. Thus, the concept of inflexibility in the modulation of fatty acid oxidation traduces an inappropriate reduction in fat oxidation during overnight fasting leading to triglyceride accumulation in the muscle, and a defective inhibition of fat oxidation during hyperinsulinaemia in accordance with the Randle cycle. In addition, the biochemical characteristics of skeletal muscle in the obese predispose to fat accumulation: citrate synthase, an enzyme of the tricarboxylic cycle activity which is a strong marker of oxidative capacity, is reduced while phosphofructokinase activity, which reflects the glycolytic potential of muscle, is increased in the obese. The ratio of phosphofructokinase/citrate synthase is a strong marker of insulin resistance. In addition, muscle lipoprotein lipase activity is decreased in obesity which is related to a decrease in whole body lipid oxidation [13]. Marker enzymes of the beta-oxidation pathway [14] and the activity of carnitine palmitoyl transferase [15] are

decreased in the obese. Thus, in obesity-related insulin resistance, the metabolic capacity of the muscle is oriented towards fatty acid esterification rather than oxidation.

3.1.2. Muscle long chain acyl CoA esters and insulin resistance

In rats, an acute elevation of plasma NEFA concentrations induces a reduction in muscle glycolysis associated initially to an increase in glycogen synthesis, followed after several hours by a decrease in glycogen synthesis [16]. During hyperinsulinaemic clamp, this metabolic situation is associated with insulin resistance, and this is accompanied by both an increase in muscle triglycerides and long chain acyl CoA (LCACoA) esters. An increase in muscle LCACoA of similar magnitude as during NEFA elevation occurred during high fat feeding in rats [17, 18]. In vitro, incubation of isolated soleus strips from rats with fatty acids for 4 h impaired their ability to take up and phosphorylate glucose. The degree of impairment depended on the type of fatty acid and insulin action correlated with the generation of LCACoA in muscle strips during incubation. In addition, when chronic high fat fed rats were given a low fat high glucose diet the day before the clamp studies, insulin resistance was reversed parallel to a decrease in LCACoA. It is noteworthy that during treatment with PPAR-γ agonists (glitazones) which reverse insulin resistance, muscle triglycerides and LCACoA decrease [19]. The following mechanisms can explain the negative effect of LCACoA on insulin sensitivity: inhibition of hexokinase, activation of protein kinase C (PKC), generation of ceramides which inhibit GLUT4 translocation, alteration of trafficking and membrane fusion of vesicles containing GLUT4, modulation of PPAR and hepatic nuclear factor 4 (HNF4) [20].

3.1.3. Liver fat storage: nonalcoholic fatty liver disease and "non alcoholic steatohepatitis"

Non alcoholic steatohepatitis (NASH) is an increasingly recognized condition frequently associated to obesity, type 2 diabetes and hyperlipidemia [21]. Among patients with NASH, 30 to 100% are obese, 10–75% are type 2 diabetics and 20–92% have dyslipidemia. The association of obesity and type 2 diabetes increases the risk and severity. Epidemiologic studies suggest that nonalcoholic fatty liver disease (NAFLD) predisposes to type 2 diabetes. In the NHANES III population (13 500 US adults aged 17–74 years) [22], subjects with NAFLD had a two times higher risk to develop type 2 diabetes than counterparts without NAFLD.

A net retention of fatty acids within hepatocytes mostly as triglycerides, precedes histologic features of NASH. Insulin resistance is the most reproducible factor associated or responsible for the development of NASH [23–25]. It is proposed that insulin resistance leads to fat accumulation via an increased lipolysis associated with hyperinsulinaemia which stimulates fatty acid reesterification and decreases their exportation by decreasing apo B100 synthesis. The progression from simple steatosis to steatohepatitis and fibrosis could result from oxidative stress. The generation of reactive oxygen species may trigger steatohepatitis and fibrosis by three mechanisms: lipid peroxydation, cytokine induction, and induction of the Fas ligand [21].

3.2. Why are lipids stored in excess out of adipose tissue?

Enlarged adipocytes are better correlated with whole body insulin resistance and are resistant to the uptake of glucose. However, these adipoctes even if fat mass is increased are unlikely to explain the whole body defect of glucose uptake because in the post-prandial state adipose tissue uptakes glucose only minimally. Data from Pima Indians show that impaired differentiation and proliferation capacity are a precipitating factor for the development of type 2 diabetes [26].

One alternative explanation for intracellular lipid accumulation is that whole body

lipid oxidation is decreased which leads to ectopic fat storage (cf. supra).

Lastly, it is possible that the pattern of adipocyte-secreted factors is different between large adipocytes and small newly differentiated adipocytes so that only large or small adipocytes are primarily implicated in the genesis of insulin resistance.

4. DESATURATION AND ELONGATION OF FATTY ACIDS AND INSULIN ACTION

Patients with obesity or type 2 diabetes are characterized by a different fatty acid composition of serum lipids as compared to healthy lean subjects [27]. This abnormal fatty acid profile is characterized by an increased proportion of palmitic (16:0) and palmitoleic (16:1 n-7) acid, low levels of linoleic acid (18:2 n-6), and a high proportion of dihomo-gamma linolenic acid (20:3 n-6). The contents of arachidonic (20:4 n-6), eicosapentaenoic (20:5 n-3) or docosahexaenoic acids (22:6 n-3) are similar between patients with insulin resistance and healthy subjects. Such an abnormal fatty acid profile in healthy subjects predicts further development of type 2 diabetes [28]. Assuming that the ratio product/precursor is an indicator of desaturase activities, the serum fatty acid pattern of insulin resistant patients traduces high activities of Δ9 and Δ6 desaturases and low activity of Δ5 desaturase [27]. Many studies have shown a positive relationship between polyunsaturated fatty acid composition of muscle phospholipids in humans and insulin sensitivity [27]. The relationship between insulin sensitivity and apparent Δ5 desaturase activity remains unclear. Among the hypotheses, dietary fatty acids could contribute perhaps in association with a genetic predisposition to modulate desaturase activity. An alternative hypothesis is that a primary defect of desaturase activity alters membrane lipid composition. Such an alteration could in turn alter membrane fluidity, the insulin signaling pathway, expression of genes or generate lipid signals (oxidized lipids or eicosanoïds) which interfere with glucose metabolism.

5. POTENTIAL BENEFICIAL EFFECTS OF LONG CHAIN N-3 FATTY ACIDS

5.1. Animal models of insulin resistance

Studies from Storlien's group have demonstrated that the substitution of fish oil (rich in n-3 long chain n-3 fatty acids) for saturated (tallow) or monoinsaturated (olive oil) or polyunsaturated n-6 (safflower oil) high fat diets (60% fat) in rats over 3 weeks completely prevented liver and muscle insulin resistance induced by the diets [16, 29–32]. We demonstrated [33] that fish oil prevents insulin resistance, when substituted for one third of the amount of safflower oil in a 60% high fat diet in rats, by preventing in the muscle both a decrease in PI3 kinase activity and a decrease in the GLUT4 level induced by the high fat diet. The mechanisms relating dietary n-3 fatty acid substitution to the protection towards insulin resistance remains in part hypothetical. The following mechanisms are likely to be implicated: (a) the decrease in muscle triglyceride content. Indeed, it has been shown that insulin sensitivity is related to muscle triglycerides in rats fed a high fat diet and that muscle triglyceride content is decreased by fish oil [34, 35]; (b) the decrease in LCFACoA as demonstrated by Neschen et al. [36]; (c) the decrease in visceral fat mass [37]; (d) the increase in unsaturation of membrane phospholipids. A strong positive relationship has been shown between the insulin sensitivity of rat muscles and the degree of unsaturation of muscle membrane phospholipids [32].

Additional data are available concerning the protective effect of n-3 LC-PUFA against high fat diet induced liver insulin resistance in rats. In rats fed a high fat diet, liver insulin resistance occurs and glucose-6-phosphatase

is overexpressed and less acutely inhibited by insulin [16, 38]. When fish oil is substituted for saturated or polyunsaturated n-6 in the high fat diet, liver insulin resistance does not occur. N-3 LC-PUFA decrease the activity and the expression of glucose-6-phosphatase, the last enzyme responsible for liver glucose output [39, 40]. Such an inhibiting effect of n-3 LC-PUFA on G6Pase activity could overcome its overactivity and help to restore normal inhibition of hepatic glucose production.

Fish oil also prevents sucrose-induced insulin resistance in rats [41–43]. However, the results are controversial as to the capacity of fish oil to reverse insulin resistance once installed after a high sucrose diet. D'Alessandro et al. [44] and Soria et al. [45] found that fish oil when substituted for vegetable oil (corn oil) in the diet reversed sucrose-induced insulin resistance. Conversely Podolin et al. [41] found that fish oil was unable to reverse insulin resistance in the same rat model. The reasons for this discrepancy are unclear. The amount of fish oil accounted for 7% of dietary calories in D'Alessandro's work [44] and for 6% in Podolin's work [41]. Thus, it is unlikely that different amounts of fish oil could explain the different effect on insulin-resistance. However the ratio of n-6/n-3 fatty acids of the diet was different because in D'Alessandro's work [44] the 7% fish oil were associated to 1% corn oil in the diet whereas in Podolin's work [41] the 6% fish oil were associated to 6% corn oil in the diet. In addition, Podolin et al. [41] used cod liver as the fish oil with a polyunsaturated-saturated (P:S) ratio of 1.23, while Podolin et al. used menhaden oil with a P:S ratio of 0.88. The duration of administration of the sucrose diet then of fish oil was different between the studies. In studies where fish oil reversed insulin resistance, a sucrose diet was given for 90 days without fish oil to induce insulin resistance then fish oil was given in addition to the sucrose diet over the next 30 days, i.e. from the 90th to the 120th day. In Podolin's work where fish oil did not reverse insulin resistance, the sucrose diet was given over 5 weeks (35 days) to induce insulin resistance then fish oil was added to the sucrose diet over the next 5 weeks (35 days). Since the duration of the high sucrose diet (15 weeks vs. 3–5 weeks) has been demonstrated to induce different degrees of insulin resistance and different metabolic changes in adipocytes [46], it can be speculated that the different duration of the sucrose diet before fish oil administration induced different metabolic responses.

5.2. Human studies

5.2.1. Healthy subjects

With regards to the preventive effect of fish oil on insulin resistance in models of diet-induced insulin resistance and on the lack of a curative effect in patients with type 2 diabetes, it was of interest to evaluate the metabolic effect of fish oil in healthy subjects. We observed [47] that a dietary substitution of 6 $g \cdot d^{-1}$ of fish oil (1.1 g EPA + 0.7 g DHA$\cdot d^{-1}$) over 3 weeks in healthy young adults studied for 6 h after a 1 $g \cdot kg^{-1}$ oral glucose load induced a 40% decrease in insulinaemic response without alteration of the glycaemic response. Plasma glucose utilization and endogenous glucose production were not modified by fish oil but whole body carbohydrate oxidation was 35% decreased, fat oxidation was 35% increased and glycogen storage 100% increased. When oral fructose was given, the insulinaemic response was also decreased by fish oil but CHO oxidation was only decreased after the 3rd h. Since fructose metabolism is mainly hepatic and independent of insulin action, the comparison of the effect of fish oil on glucose and fructose suggests that the alteration in the fuel selection resulted mainly from a different oxidative metabolism of muscle resulting from the lower insulinaemic response. Perhaps the most interesting effect was a lower insulinaemic response with regards to a similar glycaemic response which suggests that insulin sensitivity could have been increased. Following the absorption of 9 g

of the same fish oil during 24 h alone did not alter either the insulinaemic response to glucose or fuel selection (unpublished). This suggests that n-3 fatty acid incorporation in membranes is required for the metabolic effects to be observed. It is to note that Vessby et al. observed that introducing 3.6 $g \cdot d^{-1}$ of n-3 fatty acids in a high monunsaturated or saturated diet in healthy subjects did not alter insulin sensitivity [48]. n-3 LC-PUFA modulate sympathetic activity. Supplementation of the diet with DHA or fish oil for 3 months prevented young students from developing aggression against others at times of mental stress (preparation of final exams [49] and in another similar study in students submitted to the mental stress of preparing final exams decreased plasma norepinephrine concentrations) [50]. Recently, in collaboration with L. Tappy in Lausanne we observed blunting of activation of sympathetic activity during mental stress after 3 weeks of fish oil supplementation in healthy adults [51]. Overall, these results demonstrate that n-3 LC-PUFA supplementation (1.8–2 $g \cdot d^{-1}$ EPA + DHA) as dietary fish oil modulates strikingly the intermediary metabolism and sympathetic activity in a way that could help to prevent insulin resistance. However, the negative results of the KANWU study [48] suggest that the nature and the amount of other dietary fats in the diet or other unelicidated factors could be important for the observation of the positive effects of fish oil on glucose metabolism.

5.2.2. Patients with type 2 diabetes

Conversely to rats with high fat diet induced insulin resistance, fish oil dietary supplementation does not reverse insulin resistance in patients with type 2 diabetes [52] and does not ameliorate glycaemic control [53]. There is no definitive explanation for this discrepancy, but some hypotheses can be proposed. First, for unclear reasons, fish oil could have only a preventive effect on insulin resistance but not a curative effect. In accordance with this hypothesis, the observation that in rats with a high sucrose diet induced insulin resistance, fish oil prevents but does not reverse insulin resistance [41]. However, as discussed above, two other studies have shown that fish oil reverses sucrose-induced insulin resistance [44, 45].

Even though a high fat diet mimicks in many aspects insulin resistance of type 2 diabetes, some basic abnormalities may differ between the two situations. Rat metabolism is known to differ from that of human metabolism in many aspects. For example, liver PPAR-α activation in the rat strikingly stimulates peroxisomal proliferation whereas it only has a marginal effect in humans which explains that drugs such as clofibrate, a potent ligand of PPAR-α, has no deleterious proliferative effect in humans. The amount of dietary n-3 LC-PUFA may also contribute to the discrepancy observed between animal and human studies. Indeed, in most studies performed in rats, fish oil accounted for about at least 20% of the weight of the diet absorbed. Assuming a mean daily intake of 30 g of the diet by adult rats, 6 $g \cdot d^{-1}$ of fish oil is absorbed. Relatively to a mean weight of about 250 g body weight, this corresponds to an intake of 24 $g \cdot d^{-1}$ of fish oil. Such an amount is two to four times higher than the amount that has been given in type 2 diabetics. In addition, we lack animal studies evaluating the effect of low amounts of dietary n-3 fatty acids on insulin signaling.

5.2.3. Patients with nonalcoholic steatohepatitis

In rats fed a high fat diet, substitution for fish oil in the diet reduces liver triglyceride content [35, 36]. n-3 PUFA exert their effects by coordinately suppressing lipogenesis and upregulating fatty acid oxidation. The induction of lipid oxidation by n-3 PUFA in the liver results from both a reduction in the production of liver malonyl CoA driving entry and oxidation of fatty acids into mitochondria and from an induction of genes

encoding proteins involved in fatty acid oxidation and ketogenesis [54]. This effect is explained by the binding of long chain n-3 fatty acids (EPA and DHA) and/or their oxidized fatty acid derivatives to PPAR-α. The activation of PPAR-α by n-3 fatty acids results in the stimulation of genes as CPT and peroxisomal acyl-CoA oxidase [22, 55–59].

Dietary n-3 LC-PUFA inhibit lipogenesis by coordinately suppressing the expression of liver glucokinase, pyruvate kinase, glucose-6-phosphate deshydrogenase, citrate lyase, acetyl-CoA carboxylase, fatty acid synthase, stearoyl-CoA desaturase, Δ5 and Δ6 desaturases [22]. This effect of n-3 LC-PUFA is mediated by a posttranscriptional reduction in the hepatic content of SREBP-1c [60]. n-3 LC-PUFA could have a beneficial effect on NASH by inhibiting the expression and activity of stearoyl-CoA desaturase (Δ9 desaturase). Stearoyl-CoA desaturase (Δ9 desaturase) plays a major role in the secretion of VLDL by the liver as suggested by the sharp inhibition of VLDL production after the inhibition of enzymatic activity of the enzyme [61] and in mice KO for the enzyme [62]. Δ9 desaturase is regulated at the molecular level by SREBP-1c [63]. Kim et al. [64] demonstrated that fish oil downregulated Δ9 desaturase expression by inhibiting the synthesis of mRNA of SREBP-1c. Thus, the inhibition of Δ9 desaturase is with its inhibiting effect on lipogenesis and its stimulating effect on fatty acid oxidation another mechanism by which it decreases lipid synthesis and triglycerides in the liver.

In summary, n-3 LC-PUFA may have a beneficial effect to reduce liver steatosis and further steatohepatitis by decreasing malonyl-CoA and upregulating enzymes involved in liver fatty acid oxidation via PPAR-α activation on the one hand and downregulating enzymes involved in liver lipogenesis via the suppression of liver SREBP-1c on the other hand. Studies in humans should assess this potential beneficial effect of n-3 LC-PUFA.

6. CONCLUSION

In conclusion, n-3 long chain polyunsaturated fatty acids have a potential preventive effect against insulin resistance. In animal models with diet-induced insulin resistance, n-3 LC-PUFA exert their preventive effects at the level of insulin signaling in muscle (PI3-kinase activity, GLUT4 protein) and adipose tissue (GLUT4 protein). In the liver, they decrease steatosis via the inhibition of lipogenesis enzymes and Δ9 desaturase expression and activities (via a decreasing effect on SREBP-1c) and by stimulating fatty acid oxidation (via the activation of PPAR-α). They could induce a decrease in muscle intramyofibrillar triglycerides may be by reorienting fatty acids to adipose tissue via activation of PPAR-γ. Animal models of insulin resistance confirm the protective effect of n-3 LC-PUFA against insulin resistance induced by saccharose or a high fat diet. In addition some studies, but not all, also demonstrate the ability of n-3 LC-PUFA to reverse sucrose-induced insulin resistance in rats, but studies in patients with type 2 diabetes are negative. The reasons for this discrepancy remain unclear. It can be speculated that the amount of fish oil supplementation or basic different alterations of insulin signaling between animal models and type 2 diabetes play a role in this different effect of n-3 LC-PUFA. Studies in healthy subjects are encouraging in that they demonstrate that n-3 LC-PUFA could increase insulin-sensitivity at least in the post-prandial state (oral glucose load) and decrease sympathetic activity stimulation.

REFERENCES

[1] Bjornholm M, Kawano Y, Lehtihet M, Zierath JR. Insulin receptor substrate-1 phosphorylation and phosphatidylinositol 3-kinase activity in skeletal muscle from NIDDM subjects after in vivo insulin stimulation. Diabetes 1997, 46: 524–527.

[2] Cusi K, Maezono K, Osman A, Pendergrass M, Patti ME, Pratipanawatr T, DeFronzo RA,

Kahn CR, Mandarino LJ. Insulin resistance differentially affects the PI3-kinase- and MAP kinase-mediated signaling in human muscle. J Clin Invest 2000, 105: 311–320.

[3] Krook A, Bjornholm M, Galuska D, Jiang XJ, Fahlman R, Myers MG Jr, Wallberg-Henriksson H, Zierath JR. Characterization of signal transduction and glucose transport in skeletal muscle from type 2 diabetic patients. Diabetes 2000, 49: 284–292.

[4] Gaster M, Staehr P, Beck-Nielsen H, Schroder HD, Handberg A. GLUT4 is about 80% reduced in slow muscle fibers of type 2 diabetic patients: is insulin resistance in type 2 diabetes a slow, type 1 fiber disease? Diabetes 2001, 50: 1324–1329.

[5] Ryder JW, Yang J, Galuska D, Rincon J, Bjornholm M, Krook A, Lund S, Pedersen O, Wallberg-Henriksson H, Zierath JR, Holman GD. Use of a novel impermeable biotinylated photolabeling reagent to assess insulin- and hypoxia-stimulated cell surface GLUT4 content in skeletal muscle from type 2 diabetic patients. Diabetes 2000, 49: 647–654.

[6] Harris MI, Flegal KM, Cowie CC, Eberhardt MS, Goldstein DE, Little RR, Wiedmeyer HM, Byrd-Holt DD. Prevalence of diabetes, impaired fasting glucose, and impaired glucose tolerance in US adults. The Third National Health and Nutrition Examination Survey, 1988–1994. Diabetes Care 1998, 21: 518–524.

[7] Knowler WC, Pettitt DJ, Savage PJ, Bennett PH. Diabetes incidence in Pima Indians: contributions of obesity and parental diabetes. Am J Epidemiol 1981, 113: 144–156.

[8] Caro JF, Dohm LG, Pories WJ, Sinha MK. Cellular alterations in liver, skeletal muscle, and adipose tissue responsible for insulin resistance in obesity and type II diabetes. Diabetes Metab Rev 1989, 5: 665–689.

[9] Ravussin E, Smith SR. Increased fat intake, impaired fat oxidation, and failure of fat cell proliferation result in ectopic fat storage, insulin resistance, and type 2 diabetes mellitus. Ann NY Acad Sci 2002, 967: 363–378.

[10] Kelley DE, Goodpaster BH, Storlien L. Muscle triglyceride and insulin resistance. Annu Rev Nutr 2002, 22: 325–346.

[11] Goodpaster BH, Theriault R, Watkins SC, Kelley DE. Intramuscular lipid content is increased in obesity and decreased by weight loss. Metabolism 2000, 49: 467–472.

[12] Malenfant P, Joanisse DR, Theriault R, Goodpaster BH, Kelley DE, Simoneau JA. Fat content in individual muscle fibers of lean and obese subjects. Int J Obes Relat Metab Disord 2001, 25: 1316–1321.

[13] Ferraro RT, Eckel RH, Larson DE, Fontvieille AM, Rising R, Jensen DR, Ravussin E. Relationship between skeletal muscle lipoprotein lipase activity and 24-hour macronutrient oxidation. J Clin Invest 1993, 92: 441–445.

[14] Zurlo F, Nemeth PM, Choksi RM, Sesodia S, Ravussin E. Whole-body energy metabolism and skeletal muscle biochemical characteristics. Metabolism 1994, 43: 481–486.

[15] Simoneau JA, Veerkamp JH, Turcotte LP, Kelley DE. Markers of capacity to utilize fatty acids in human skeletal muscle: relation to insulin resistance and obesity and effects of weight loss. FASEB J 1999, 13: 2051–2060.

[16] 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: 1768–1774.

[17] Ellis BA, Poynten A, Lowy AJ, Furler SM, Chisholm DJ, Kraegen EW, Cooney GJ. Long-chain acyl-CoA esters as indicators of lipid metabolism and insulin sensitivity in rat and human muscle. Am J Physiol Endocrinol Metab 2000, 279: E554–E560.

[18] Oakes ND, Bell KS, Furler SM, Camilleri S, Saha AK, Ruderman NB, Chisholm DJ, Kraegen EW. 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, 12: 2022–2028.

[19] Ye JM, Doyle PJ, Iglesias MA, Watson DG, Cooney GJ, Kraegen EW. Peroxisome proliferator-activated receptor (PPAR)-alpha activation lowers muscle lipids and improves insulin sensitivity in high fat-fed rats: comparison with PPAR-gamma activation. Diabetes 2001, 50: 411–417.

[20] Cooney GJ, Thompson AL, Furler SM, Ye J, Kraegen EW. Muscle long-chain acyl CoA esters and insulin resistance. Ann NY Acad Sci 2000, 967: 196–207.

[21] Angulo P. Non alcoholic fatty liver disease. N Engl J Med 2002, 346: 1221–1231.

[22] Clark JM, Diehl AM. Nonalcoholic fatty liver disease and the risk of type 2 diabetes in the United States. Diabetes 2001, 50: A38.

[23] Marchesini G, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, Forlani G, Melchionda N. Association of nonalcoholic fatty liver disease with insulin resistance. Am J Med 1999, 107: 450–455.

[24] Ryysy L, Hakkinen AM, Goto T, Vehkavaara S, Westerbacka J, Halavaara J, Yki-Jarvinen H. Hepatic fat content and insulin action on free fatty acids and glucose metabolism rather

than insulin absorption are associated with insulin requirements during insulin therapy in type 2 diabetic patients. Diabetes 2000, 49: 749–758.

[25] Virkamaki A, Korsheninnikova E, Seppala-Lindroos A, Vehkavaara S, Goto T, Halavaara J, Hakkinen AM, Yki-Jarvinen H. Intramyocellular lipid is associated with resistance to in vivo insulin actions on glucose uptake, antilipolysis, and early insulin signaling pathways in human skeletal muscle. Diabetes 2001, 50: 2337–2343.

[26] Weyer C, Foley JE, Bogardus C, Tataranni PA, Pratley RE. Enlarged subcutaneous abdominal adipocyte size, but not obesity itself, predicts type II diabetes independent of insulin resistance. Diabetologia 2000, 43: 1498–1506.

[27] Vessby B, Gustafsson IB, Tengblad S, Boberg M, Andersson A. Desaturation and elongation of fatty acids and insulin action. Ann NY Acad Sci 2002, 967: 183–195.

[28] Vessby B, Aro A, Skarfors E, Berglund L, Salminen I, Lithell H. The risk to develop NIDDM is related to the fatty acid composition of the serum cholesterol esters. Diabetes 1994, 43: 1353–1357.

[29] Kraegen EW, Clark PW, Jenkins AB, Daley EA, Chisholm DJ, Storlien LH. In vivo insulin resistance in individual peripheral tissues of the high fat fed rat: assessment by euglycemic clamp plus deoxyglucose administration. Diabetologia 1986, 29: 192–198.

[30] Kraegen EW, Clark PW, Jenkins AB, Daley EA, Chisholm DJ, Storlien LH. Development of muscle insulin resistance after liver insulin resistance in high fat fed rats. Diabetes 1991, 40: 1397–1403.

[31] Storlien LH, Kraegen EW, Chisholm DJ, Ford GL, Bruce DG, Pascoe WS. Fish oil prevents insulin resistance induced by high fat feeding in rats. Science 1987, 237: 885–888.

[32] Storlien LH, Jenkins AB, Chisholm DJ, Pascoe WS, Khouri S, Kraegen EW. Influence of dietary fat composition on development of insulin resistance in rats. Diabetes 1991, 40: 280–289.

[33] 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 Endocrinol Metab 2002, 282: E664–E671.

[34] Jucker BM, Cline GW, Barucci N, Shulman GI. Differential effects of safflower oil versus fish oil feeding on insulin-stimulated glycogen synthesis, glycolysis, and pyruvate dehydrogenase flux in skeletal muscle: a ^{13}C nuclear magnetic resonance study. Diabetes 1999, 48: 134–140.

[35] Simoncikova P, Wein S, Gasperikova D, Ukropec J, Certik M, Klimes I, Sebokova E. Comparison of the extrapancreatic action of gamma-linolenic acid and n-3 PUFAs in the high fat diet-induced insulin resistance. Endocr Regul 2002, 36: 143–149.

[36] Neschen S, Moore I, Regittnig W, Yu CL, Wang Y, Pypaert M, Petersen KF, Shulman GI. Contrasting effects of fish oil and safflower oil on hepatic peroxisomal and tissue lipid content. Am J Physiol Endocrinol Metab 2002, 282: E395–E401.

[37] Kim JY, Nolte LA, Hansen PA, Han DH, Ferguson K, Thompson PA, Holloszy JO. High-fat diet-induced muscle insulin resistance: relationship to visceral fat mass. Am J Physiol Regul Integr Comp Physiol 2000, 279: R2057–R2065.

[38] Commerford SR, Ferniza JB, Bizeau ME, Thresher JS, Willis WT, Pagliassotti MJ. Diets enriched in sucrose or fat increase gluconeogenesis and G-6-Pase but not basal glucose production in rats. Am J Physiol Endocrinol Metab 2002, 283: E545–E555.

[39] Daniele N, Bordet JC, Mithieux G. Unsaturated fatty acids associated with glycogen may inhibit glucose-6 phosphatase in rat liver. J Nutr 1997, 127: 2289–2292.

[40] Rajas F, Gautier A, Bady I, Montano S, Mithieux G. Polyunsaturated fatty acyl coenzyme A suppress the glucose-6-phosphatase promoter activity by modulating the DNA binding of hepatocyte nuclear factor 4 alpha. J Biol Chem 2002, 277: 15736–15744.

[41] 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–R848.

[42] Luo J, Rizkalla SW, Boillot J, Alamowitch C, Chaib H, Bruzzo F, Desplanque N, Dalix AM, Durand G, Slama G. 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: 1951–1958.

[43] Peyron-Caso E, Fluteau-Nadler S, Kabir M, Guerre-Millo M, Quignard-Boulange A, Slama G, Rizkalla SW. 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: 360–366.

[44] D'Alessandro ME, Lombardo YB. Duration of feeding on a sucrose-rich diet determines metabolic and morphological changes in rat adipocytes. J Appl Physiol 2001, 91: 2109–2116.

[45] Soria A, Chicco A, Eugenia D'Alessandro M, Rossi A, Lombardo YB. Dietary fish oil reverse epididymal tissue adiposity, cell hypertrophy and insulin resistance in dyslipemic sucrose fed rat model small star, filled. J Nutr Biochem 2002, 13: 209–218.

[46] Soria A, D'Alessandro ME, Lombardo YB. Duration of feeding on a sucrose-rich diet determines metabolic and morphological changes in rat adipocytes. J Appl Physiol 2001, 91: 2109–2116.

[47] Delarue J, Couet C, Cohen R, Brechot JF, Antoine JM, Lamisse F. Effects of fish oil on metabolic responses to oral fructose and glucose loads in healthy humans. Am J Physiol 1996, 270: E353–E362.

[48] Vessby B, Unsitupa M, Hermansen K, Riccardi G, Rivellese AA, Tapsell LC, Nalsen C, Berglund L, Louheranta A, Rasmussen BM, Calvert GD, Maffetone A, Pedersen E, Gustafsson IB, Storlien LH. Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study. Diabetologia 2001, 44: 312–319.

[49] Hamazaki T, Sawazaki S, Itomura M, Asaoka E, Nagao Y, Nishimura N, Yazawa K, Kuwamori T, Kobayashi M. The effect of docosahexaenoic acid on aggression in young adults. A placebo-controlled double-blind study. J Clin Invest 1996, 97: 1129–1133.

[50] Hamazaki T, Sawazaki S, Nagasawa T, Nagao Y, Kanagawa Y, Yazawa K. Administration of docosahexaenoic acid influences behavior and plasma catecholamine levels at times of psychological stress. Lipids Suppl 1999, 34: S33–S37.

[51] Delarue J, Matzinger O, Binnert C, Schneiter P, Chiolero R, Tappy L. Fish oil prevents the adrenal activation elicited by mental stress in healthy men. Diabetes Metab 2003, 29: 289–295.

[52] Rivellese AA, De Natale C, Lilli S. Type of dietary fat and insulin resistance. Ann NY Acad Sci 2002, 967: 329–335.

[53] Montori VM, Farmer A, Wollan PC, Dinneen SF. Fish oil supplementation in type 2 diabetes: a quantitative systematic review. Diabetes Care 2000, 23: 1407–1415.

[54] Jump DB, Clarke SD. Regulation of gene expression by dietary fat. Annu Rev Nutr 1999, 19: 63–90.

[55] Hashimoto T, Cook WS, Qi C, Yeldandi AV, Reddy JK, Rao MS. Defect in peroxisome proliferator-activated receptor alpha-inducible fatty acid oxidation determines the severity of hepatic steatosis in response to fasting. J Biol Chem 2000, 275: 28918–28928.

[56] Kersten S, Seydoux J, Peters JM, Gonzalez FJ, Desvergne B, Wahli W. Peroxisome proliferator-activated receptor alpha mediates the adaptive response to fasting. J Clin Invest 1999, 103: 1489–1498.

[57] Leone TC, Weinheimer CJ, Kelly DP. A critical role for the peroxisome proliferator-activated receptor alpha (PPARalpha) in the cellular fasting response: The PPARalpha-null mouse as a model of fatty acid oxidation disorders. Proc Natl Acad Sci USA 1999, 96: 7473–7478.

[58] Reddy JK. Nonalcoholic steatosis and steatohepatitis. III. Peroxisomal beta-oxidation, PPAR alpha, and steatohepatitis. Am J Physiol Gastrointest Liver Physiol 2001, 81: G1333–G1339.

[59] Clarke SD. Nonalcoholic steatosis and steatohepatitis. I. Molecular mechanism for polyunsaturated fatty acid regulation of gene transcription. Am J Physiol Gastrointest Liver Physiol 2001, 281: G865–G869.

[60] Xu J, Nakamura MT, Cho HP, Clarke SD. Sterol regulatory element binding protein-1 expression is suppressed by dietary polyunsaturated fatty acids. A mechanism for the coordinate suppression of lipogenic genes by polyunsaturated fats. J Biol Chem 1999, 274: 23577–23583.

[61] Legrand P, Catheline D, Fichot MC, Lemarchal P. Inhibiting Δ9-desaturase activity impairs triacylglycerol secretion in cultured chicken hepatocytes. J Nutr 1997, 127: 249–256.

[62] Miyazaki M, Kim Y, Gray-Keller MP, Attie AD, Ntambi JM. The biosynthesis of hepatic cholesterol esters and triglycerides is impaired in mice with a disruption of the gene for stearoyl-CoA desaturase 1. J Biol Chem 2000, 275: 30132–30138.

[63] Shimano H, Yahagi N, Amemiya-Kudo M, Hasty AH, Osuga J, Tamura Y, Shionoiri F, Iizuka Y, Ohashi K, Harada K, Gotoda T, Ishibashi S, Yamada N. Sterol regulatory element-binding protein-1 as a key transcription factor for nutritional induction of lipogenic enzyme genes. J Biol Chem 1999, 274: 35832–35839.

[64] Kim H, Takahashi M, Ezaki O. Fish oil feeding decreases mature SREBP-1 by down-regulation of SREBP-1c mRNA in mouse liver. J Biol Chem 1999, 274: 25892–25898.