Proceedings of the Nutrition Society (2020), **79**, 417–427
doi:10.1017/S0029665120000087
 First published online 6 March 2020

The 13th European Nutrition Conference (FENS 2019) was held at the Convention Centre Dublin, Ireland on 15–18 October 2019

Conference on 'Malnutrition in an Obese World: European Perspectives' Symposium 3B: Omega-3 fatty acids: from lab to clinic

Are marine *n*-3 fatty acids protective towards insulin resistance? From cell to human

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Marine *n*-3 fatty acids improve most of the biochemical alterations associated with insulin resistance (IR). Experimental models of dietary-induced IR in rodents have shown their ability (often at a very high dose) to prevent IR, but with sometimes a tissue specific effect. However, in a high sucrose diet-induced IR rat model, they are unable to reverse IR once installed; in other rodent models (dexamethasone, Zucker rats), they are inefficacious perhaps because of the severity of IR. The very low incidence of type-2 diabetes (T2D) in Inuits in the 1960s, which largely increased over the following decades in parallel to the replacement of their traditional marine food for a western diet strongly suggests a protective effect of marine *n*-3 towards the risk of T2D; this was confirmed by reversal of its incidence in intervention studies reintroducing their traditional food. In healthy subjects and insulin-resistant non-diabetic patients, most trials and meta-analyses conclude to an insulin-sensitising effect and to a very probable preventive or alleviating effect towards IR. Concerning the risk of T2D, concordant data allow us to conclude the protective effect of marine *n*-3 in Asians while suspicion exists of an aggravation of risk in Westerners, but with the possibility that it could be explained by a high heterogeneity of studies performed in this population. Some longitudinal cohorts in US/European people showed no association or a decreased risk. Further studies using more homogeneous doses, sources of *n*-3 and assessment of insulin sensitivity methods are required to better delineate their effects in Westerners.

***n*-3 Fatty acids: Type-2 diabetes: Inflammation: Adipose tissue**

Pathophysiology of insulin resistance

In this section, we will briefly summarise the main mechanisms of insulin resistance (IR). The main tissues where insulin exerts its action are the liver, skeletal muscle and adipose tissue (AT). After binding of insulin to its receptor, the first step is the autophosphorylation of the β subunit. The insulin receptor tyrosine kinase activation recruits and phosphorylates several substrates including insulin receptor substrates (mainly insulin receptor substrates 1 and 2). These substrates recruit and activate phosphatidylinositol 3′-kinase (PI3K). Activation of PI3K generates phosphatidylinositol (3,4,5) triphosphate activating 3-phosphoinositide-dependent protein kinase-1 and mammalian target of rapamycin complex 2, which mediate the metabolic effects of insulin. 3-Phosphoinositide-dependent protein kinase-1 and mammalian target of rapamycin complex 2 in turn activate protein kinases

Abbreviations: AT, adipose tissue; ChREBP, carbohydrate response element binding protein; FFAR4, NEFA receptor-4; GPR, G protein-coupled receptor; HFD, high fat diet; HOMA-IR, homoeostatic model assessment-insulin resistance; IR, insulin resistance; NLRP3, nucleotide-binding oligomerisation domain-like receptor (NLR) family, pyrin domain containing 3; MyD88, myeloid differentiation primary response gene 88; PI3K, phosphatidylinositol 3′-kinase; RCT, randomised controlled trial; RR, relative risk; SREBP-1c, sterol-regulatory element-binding protein-1c; T2D, type-2 diabetes.

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Akt (Akt1 and Akt2). Akt phosphorylates downstream targets including Bad (important for cell survival), Gsk3 (regulating growth and glycogen synthesis), AS160 (GLUT4 translocation) and FOXO1 (controlling hepatic gluconeogenesis and adipocyte differentiation)[1,2].

In patients with non-communicable diseases, IR results from a polygenic predisposition[3] associated with sedentariness and overeating. In overweight subjects, loss of expendability of AT[4] and/or excess of visceral adiposity promote an increase in release of NEFA leading to an ectopic storage of TAG mainly in the hepatocyte, muscle cell and pancreatic β cell[5–7]. The *in situ* release from ectopic stored TAG, of long-chain acyl-CoA and their metabolites, in particular diacylglycerols[8] and ceramides[9] and acyl-carnitines interfere with the insulin-signalling pathway by activating some species of protein kinase C. These alterations have been called lipotoxicity. In addition, macrophages and other immune cells infiltrate the AT, releasing pro-inflammatory cytokines leading to an inflammation of AT and beyond to other tissues, which induces IR *in situ*[10,11].

In muscle, mitochondrial dysfunction (reduction in mitochondrial phosphorylation and oxidation activity and decrease in mitochondria's density) also contributes to IR by promoting myofibrillar accumulation of fatty acids by a decrease in their oxidation[12].

In liver, excess storage of TAG, resulting from increased de novo lipogenesis and/or re-esterification of NEFA, induces IR via lipotoxicity.

In AT, inflammation leads to IR *in situ*, which increases the release of NEFA by two main mechanisms: (a) decrease in activation of carbohydrate response element binding protein (ChREBP)[13] leading in turn to a less activation of adipocyte de novo lipogenesis and re-esterification of NEFA released by lipolysis and (b) decrease in inhibition of lipolysis by insulin[14,15].

Other mechanisms of IR have been described: endoplasmic reticulum stress, reactive oxygen intermediates, competition between fatty acids and glucose[16].

The interest in the potential protective effects of marine *n*-3 towards IR arose from the epidemiological observation in the 1960s of the virtual absence of diabetes in Greenland and Alaskan Eskimos, populations consuming a huge amount of marine products rich in marine *n*-3[17–19], and from the pioneering work of Storlien *et al.*[20] demonstrating that a high dose of fish oil prevented in rats the high fat diet (HFD)-induced IR. Since then, a considerable amount of experimental, epidemiological, physiological studies, randomised controlled trials (RCT), meta-analyses and reviews have been published on this topic.

Effects of marine *n*-3 fatty acids towards insulin resistance: basic mechanisms

Marine *n*-3 modulate insulin sensitivity via several targets: anti-inflammatory effects, PPARα and PPARγ, sterol-regulatory element-binding protein 1c (SREBP-1c), ChREBP, NF-κB, miRNA, endoplasmic reticulum stress, NEFA receptor-4 (FFAR4)/G protein-coupled receptor (GPR) 120 and mitochondrial function.

Biochemical mechanisms of the anti-inflammatory effects in adipose tissue

One major target of the effects of marine *n*-3 towards IR is inflammation of AT of obese people. Thus, it is useful to describe briefly the mechanisms of their anti-inflammatory effects, which have been recently reviewed in detail by Calder[21]. Briefly, they incorporate, in a time and dose-dependent manner, into phospholipids of the membrane of immune cells (mononuclear cells and macrophages), partly replacing arachidonic acid, leading to a decrease in the production of its pro-inflammatory mediators, such as hydroxyeicosatetraenoic acid, 2-series prostaglandins, leucotriene B4 and thromboxanes. In addition, they decrease the expression of cyclooxygenase 2 (COX-2 gene) amplifying the decrease in 2-series prostaglandins. EPA is, as arachidonic acid, a substrate for cyclooxygenase, lipoxygenase and cytochrome P450 but, at odds with arachidonic acid, the eicosanoids produced, such as leucotriene B5, have a weak inflammatory effect because eicosanoid receptors have a lower affinity for EPA-produced eicosanoids than arachidonic acid-produced ones.

Marine *n*-3 also decrease the production of pro-inflammatory cytokines (TNF-α, IL-1 and IL6) in response to lipopolysaccharide, as well as the production of adhesion molecules such as vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 on the surface of endothelial cells and monocytes. All these anti-inflammatory effects can be at least partly explained by their impact on NF-κB signalling. NF-κB is activated by extracellular inflammatory stimuli, which induces the phosphorylation of the inhibitory subunit of NF-κB (IκB) allowing the translocation of the remaining NF-κB dimer to the nucleus, which up-regulates inflammatory gene expression. EPA and DHA inactivation of NF-κB signalling is associated with inhibition of IκB phosphorylation. Lipopolysaccharide plays a critical role in ligand recognition and receptor activation for Toll-like receptors 4 and 2. Myeloid differentiation primary response gene 88 (MyD88) is the canonical adaptor for the early stages of the signalling cascade that activates NF-κB signalling pathways downstream of members of Toll-like receptor and IL-1 receptor families. DHA acts upstream of MyD88 because it does not inhibit NF-κB cascade in absence of MyD88[22]. Toll-like receptor 4, MyD88 and other signalling proteins associate into lipid rafts in inflammatory cells exposed to lipopolysaccharide. DHA, and perhaps EPA, prevents, in inflammatory cells, recruitment of these proteins into lipid rafts[23].

Marine *n*-3 also inhibit NF-κB by binding to PPARγ[24] and to FFAR4/GPR120[25]. PPAR and GPR120/GPR40 signalling are also required for marine *n*-3 fatty acid inhibition of the nucleotide-binding oligomerisation domain-like receptor (NLR); NLR family, pyrin domain containing 3 (NLRP3) inflammasome via activation of β-arrestin-2, a downstream scaffold protein of GPR120/GPR40, which binds to NLRP3[26,27]. The inhibition of the NLRP3 inflammasome by EPA and DHA has been demonstrated in macrophage cell lines as well as in primary human and mouse macrophages. EPA and DHA inhibit the NLRP3 inflammasome by

https://doi.org/10.1017/S0029665120000087 Published online by Cambridge University Press

inhibiting NF-κB activation[28]. EPA and DHA also reduce inflammasome gene expression[29].

Another major way for EPA and DHA to decrease inflammation is to produce the so-called specialised pro-resolving lipid mediators[30]. These specialised pro-resolving lipid mediators include E-resolvins produced from EPA and D-resolvins, maresins and protectins produced from DHA. Recently, resolvins produced from docosapentaenoïc acid have also been identified[31]. Specialised pro-resolving lipid mediators play a major role as anti-inflammatory and inflammation resolving molecules. They inhibit *trans*-endothelial migration of leucocytes and decrease the production of pro-inflammatory cytokines. They exert their biological effects as agonists of receptors of GPCR family (ALX/FPR2, DRV1/GPR32, DRV2/GPR18, ERV1/cemR23) and as antagonists to receptors of pro-inflammatory eicosanoids (BLT1)[31].

The anti-inflammatory effects of marine *n*-3 fatty acids, by improving AT function, plays a major role in their protective effect towards IR in obese[32]. In AT, EPA and DHA bind to PPARγ and induce the secretion of adiponectin, an adipokine with anti-inflammatory properties[33]. Plasma adiponectin concentrations are increased following marine *n*-3 fatty acids consumption in both obese rodent models and obese human subjects (1·8 g/d EPA for 3 months)[34]. They reduce the expression of TNF-α, IL-6, macrophage chemoattractant protein 1 and plasminogen activator inhibitor-1[35], bind to FFAR4/GPR120 inhibiting NF-κB and c-Jun amino-terminal kinase signalling cascade[25]. In HFD-fed mice, protectin D1 is lacking associated with AT inflammation and IR. When the same HFD is given to fat-1 mice, a model of transgenic endogenous synthesis of *n*-3 fatty acids, protectin-D1 is restored in AT, which prevented obesity-linked inflammation and IR. This was observed without alteration of food intake, weight gain or adiposity[36]. In addition, in HFD db/db mice, EPA and DHA (30 % of energy from safflower oil with 40 % of oil volume being replaced by a concentrate of highly purified EPA and DHA re-esterified to TAG for 6 weeks) completely prevented AT macrophages infiltration[37]. FFAR4/GPR120 is a key player as mediator of this effect[25]. A shift in AT macrophage polarisation from M1 to a M2 phenotype has been reported with DHA (4 μg/g intraperitoneally for 10 d) in HFD-fed mice[38]. This shift from M1 to M2 macrophage phenotype has also been shown in transgenic fat-1 mice[39]. Supplementation with 4 g EPA + DHA ethyl esters/d for 12 weeks in obese human subjects decreased M1 macrophage infiltration in AT and pro-inflammatory macrophage chemoattractant protein 1 in AT and plasma[40]. The supplementation with 3·36 g/d EPA and DHA for 8 weeks in severely obese patients decreased in AT the expression of markers of M1 phenotype and increased this of M2 phenotype[41].

The biological effects of marine *n*-3 fatty acids in AT other than their anti-inflammatory effects have been extensively reviewed elsewhere and will not been presented in the current paper[42–44].

We showed, in rats, that marine *n*-3 may improve insulin signalling in AT through the regulation of the expression or the translocation of the insulin-dependent GLUT4, which in turn could stimulate glucose uptake[45,46]. This was also observed in several *in vitro* and *in vivo* studies in rodents as reviewed by Martínez-Fernández *et al.*[42]. A stimulating effect of marine *n*-3 fatty acids is strongly suggested by the observation that *n*-3-depleted rats had a lower basal and insulin-stimulated AT glucose uptake[47].

Biochemical mechanisms of alleviation of insulin resistance in liver

As discussed earlier, IR in the liver of obese people is mainly determined by the lipotoxicity resulting primarily from the ectopic storage of fatty acids inside hepatocytes. Marine *n*-3 can alleviate liver IR by altering the expression and nuclear localisation of transcription factors, inducing the activity of genes encoding the activity of *de novo* lipogenesis (SREBP-1c and ChREBP/MLX (ChREBP and its heterodimer partner Max-like factor-X) on one hand and of fatty acid oxidation (PPARα) on the other hand. The enzymes of de novo lipogenesis whose genes are controlled by SREBP-1c and ChREBP/MLX are: ATP citrate lyase, acetylCoA carboxylase and fatty acid synthase. The enzyme of glycolysis whose gene is controlled by ChREBP/MLX is L-pyruvate kinase.

EPA and DHA inhibit the nuclear translocation of ChREBP[48], and reduce mRNA and active protein expression of SREBP-1c[49–51]. More details can be found in recent reviews[44,52].

These effects contribute to reduce lipotoxicity by reducing the accumulation of fatty acids and consequently of their metabolites (ceramides and diacylglycerols) within hepatocytes.

EPA and DHA also stimulate fatty acid oxidation by binding directly to the PPARα, a fatty acid-regulated nuclear receptor. PPARα regulates gene expression in association with the retinoid X receptor. Marine *n*-3 bind to and activate PPARα, which results in increased expression of key enzymes involved in fatty acid oxidation in mitochondria, peroxisomes and microsomes, carnitine palmitoyl transferase 1, acyl CoA oxidase and cytochrome P450 4A, respectively.

EPA and DHA also increase TAG catabolism[53] by increasing hepatic abundance of atriglyceride lipase (mRNA and protein) and CGI58 (protein). Atriglyceride lipase and CGI58 act together with perilipin, hormone sensitive lipase, monoacyl glycerolipase to promote the hydrolysis of TAG. This effect involves PPARβ[53].

Recently, it has been shown that marine *n*-3 decreased liver steatosis by activating FFAR4 (GPR120) in hepatocytes; its signalling cascade sequentially involves FFAR4, Gq/11 proteins, Ca^{2+}/calmodulin-dependent protein kinase kinase, AMP kinase and SREBP-1c suppression (RNA, protein)[54].

More details can be found in recent reviews[44,52]. However, one study showed that *Ffar4* knockouts and heterozygous mice fed a high fat, high sucrose *n*-3 PUFA diet for 36 weeks were protected against obesity, hepatic TAG accumulation and whole-body IR induced by the high fat, high sucrose diet, demonstrating that

https://doi.org/10.1017/S0029665120000087 Published online by Cambridge University Press

FFAR4 signalling was not required for the insulin-sensitising effects mediated by *n*-3 PUFA[55].

Biochemical mechanisms of alleviation of insulin resistance in muscle

We showed in rats fed a HFD (60 % safflower oil) that a high dose of fish oil (20 % fish oil) completely prevented the HFD-induced decrease in PI3K activity and GLUT4 content in muscle[45]. However, when rats were given a low dose of fish oil (2·2 % of energy inside a low fat diet 6·6 % of energy as peanut–rape oil), we observed a decrease in PI3K activity in muscle while the early steps of insulin signalling cascade as well as GLUT4 content remained unaltered[46]. In the same study, dexamethasone-induced IR was not prevented and fish oil amplified the dexamethasone-induced decrease in PI3K activity, without being able to prevent IR. It is possible that the dose of dexamethasone used was too high to be counteracted by this low dose of fish oil. By expressing the PI3K/phosphatase and tensin homologue/Akt pathway in a yeast-based model, we observed that long-chain *n*-3 PUFA were able to alleviate the overexpression of PI3K[56]. As the inhibition of mammalian PI3K was expressed in an exogenous cellular context in yeast, the effect of *n*-3 PUFA was likely to be a direct effect on PI3K. Other studies in rats have confirmed the beneficial effect of marine *n*-3 on insulin signalling in muscle of HFD-fed rats[57,58], or high sucrose-fed rats[59].

Marine *n*-3 also decrease inflammation in muscle of HFD-fed rats (22 % fish oil inside a 40 % fat diet)[58], the effect being observed with DHA but not with EPA (3·2 % of total fat)[60]. DHA (30 μM) prevented IR in C2C12 myotubes exposed to palmitate (500 μM) by decreasing protein kinase C-θ activation and restoring cellular acylcarnitine profile, insulin-dependent Akt phosphorylation and glucose uptake[61].

Marine *n*-3 may also alleviate muscle IR by decreasing the mRNA and proteins associated with endoplasmic reticulum stress as shown in myotubes[62,63]. In this latter study, oxidative stress in muscle was also reduced. An anti-inflammatory effect of DHA has also been shown in C2C2 myotubes exposed to palmitate or lipopolysaccharide resulting in the prevention of the increase in prostaglandin-endoperoxide synthase 2, IL-6 and TNF-α mRNA level, probably through the inhibition of p38 mitogen-activated protein kinase and c-Jun amino-terminal kinase[61].

Marine n-*3 fatty acids prevent dietary-induced insulin resistance in rodent models*

The pioneering work was carried out by Storlien *et al.* which merits a description[20]. Male adult Wistar rats were separated in three groups fed for 31 d a chow diet containing 12 % fat or a HFD containing 59 % fat (safflower oil) or a HFD containing 39 % safflower oil plus 20 % fish (tuna) oil (HFDFO), which represented a change of 6 % of safflower oil fatty acids by marine *n*-3. Whole-body insulin sensitivity was assessed by insulin clamp and radioactive tracers of glucose. Insulin action in several tissues was also assessed by using the non-metabolisable 2-deoxy glucose. Whole-body glucose disposal was reduced by 52 % and hepatic glucose production was less inhibited during hyperinsulinaemia with high fat feeding. These alterations were completely prevented in HFDFO-fed rats. HFDFO also prevented the HFD-induced alterations of the net glycolytic flux and glycogen storage. It is to note that AT glucose uptake was not affected by HFD but that fish oil feeding reduced insulin-stimulated glucose metabolism in white AT. The authors were cautious about an extrapolation to human insulin resistance because the amount of marine *n*-3 fatty acids used represented a large intake equivalent to 8–9 g/d in human subjects.

Since that work several others using HFD[45] or high sucrose diet feeding[64,65] have confirmed the protecting effect of marine *n*-3 fatty acids towards IR as reviewed[44,66].

However, other studies have failed to demonstrate the ability of marine *n*-3 to prevent IR in rodents. As discussed earlier, a low dose of fish oil (2·2 % of energy) was unable to prevent dexamethasone-induced IR as assessed by an oral glucose tolerance test or glycaemic response to an intra-peritoneal insulin injection[46,67]. Gilliam *et al.* showed that *n*-3 (10 % menhaden oil, *n*-3: 19 % of total fatty acid intake) failed to reduce IR in Zucker obese rats[68]. One other major point is that if marine *n*-3 are able to prevent IR, they are unable to reverse it when installed as demonstrated by Podolin *et al.*[64] in a model of sucrose-induced IR in rats. This may be of crucial importance to explain the discrepancy in human subjects when *n*-3 are not able to reverse IR of patients with type-2 diabetes (T2D).

Effects of marine *n*-3 on insulin sensitivity in human subjects

Epidemiologic studies in Inuits

Among the Inuits of Alaska, the prevalence of diabetes in 1957 in adults aged over 35 years was extremely low (0·6 to 1·6/1000/year). Among the Eskimos of Greenland, the incidence in 1962 was 0·6/1000/year. In the Eskimos living in the Upernavik district,[69] over the period 1950–1974, only one case of diabetes was reported for nine cases observed in the Danish population. At the beginning of the 1980s, the prevalence of diabetes among Inuits was 4/1000/year in Canada, and 0/1000/year in Russian; in 1987, it was 47/1000/year in Alaska[70]. Between 1999 and 2002, the prevalence had reached 100/1000/year in Greenlanders Inuits[71]. In adults, aged 20–79, it was 1·41 % in 2008, 1·85 % in 2010, 2·29 % in 2012[72] and 2·36 % in 2014[73].

This very significant increase in the prevalence of diabetes is explained by the sedentarisation and the westernisation. This change of lifestyle was accompanied by the increase in the prevalence of obesity and metabolic syndrome[74]. Several intervention studies have concluded the protective effect of the traditional Inuit food pattern rich in marine *n*-3 towards T2D and metabolic syndrome in this population[75–80].

https://doi.org/10.1017/S0029665120000087 Published online by Cambridge University Press

Physiological studies of insulin sensitivity in human subjects

While biochemical and physiological data in rodent models and epidemiologic studies in Inuits highlight a protective effect of marine *n*-3 towards IR, the physiological data in healthy or insulin-resistant human subjects (including patients with T2D) are more contrasted.

Healthy subjects

In healthy young subjects, we showed, by using a double stable isotopes labelled glucose method, that 6 g/d fish oil (1·8 g/d EPA + DHA over 3 weeks) decreased by about 40 % the insulin response to an oral glucose or fructose load without altering either whole body glucose disposal or inhibition of hepatic glucose production, which strongly suggested an increasing effect on insulin sensitivity[81]. In a further study, we showed that the same dose of fish oil given over 6 weeks prior to an oral low dose of dexamethasone aiming to induce an experimental IR, also reduced the dexamethasone-induced hyperinsulinaemic response following an oral glucose load without altering either whole body glucose disposal or inhibition of hepatic glucose production, which suggested a partial prevention of the dexamethasone-induced IR[82]. However, with a lower fish oil dosage (850 mg/d EPA + DHA over 6 weeks), by using a three-step hyperinsulinaemic euglycaemic clamp technique and labelled glucose, we found that dexamethasone-induced IR in liver and muscle was amplified[83]. Faeh *et al.*[84] found that a 6-d high-fructose diet (corresponding to an extra 25 % of total energy) in healthy volunteers induced hypertriglyceridaemia and liver IR without altering whole body glucose disposal. A fish oil supplementation (1·8 g/d EPA + DHA over 28 d) reversed dyslipidaemia but not liver IR.

Insulin-resistant subjects

Lalia *et al.*[85] studied in a cross-over design overweight (BMI > 25 kg/m^2) non-diabetic insulin-resistant subjects (homoeostatic model assessment-insulin resistance (HOMA-IR) < 2·6) receiving 3·9 g/d EPA + DHA over 6 months *v.* placebo. They found that *n*-3 had no effects on whole body glucose disposal but modestly improved liver IR. Albert *et al.*[86] studied overweight men (BMI 25–30 kg/m^2) aged 35–55 years with no diabetes. Insulin sensitivity was assessed by a 75 g oral glucose load by using the Matsuda and DeFronzo method[87]. The subjects were separated according to their *n*-3 index (higher tertile *v.* lower tertile). Increasing *n*-3 index was correlated with higher insulin sensitivity and that one was 43 % higher in those with a higher tertile than in those with a lower tertile. Clark *et al.*[88] included males and females aged 40–61 years and had at least one of the following: impaired fasting glucose, impaired glucose tolerance or T2D with newly diagnosed (HbA1c < 7 % and not requiring hypoglycaemic therapy). Insulin sensitivity was assessed by a hyperinsulinaemic–euglycaemic–euaminoacidaemic clamp in two parallel randomised double-blind group receiving 6 g fish oil (3·9 g/d EPA + DHA) or 6 g/d maize oil for 9 months. No effect of marine *n*-3 was observed on whole body glucose disposal and hepatic glucose production. In insulin-resistant haemodialised patients, we found no effect of fish oil (1·8 g/d EPA + DHA over 3 weeks) on insulin sensitivity assessed by a dual labelling of an oral glucose load, but sympathetic over activity was significantly improved[89].

In summary, the results depend on the severity of IR. It can be concluded that marine *n*-3 have no effect on patients with severe IR (T2D or haemodialised) but an insulin-sensitising effect in subjects with moderate IR (overweight).

Patients with type-2 diabetes

Several RCT have used the hyperinsulinaemic–euglycaemic clamp to evaluate the effect of short term (<8 weeks) marine *n*-3 (mean 3 g EPA + DHA/d) supplementation on IR. They are all concordant in finding no effect on whole body glucose disposal and hepatic glucose production reflecting no improvement of liver and muscle IR[90–97]. This is in accordance with the work of Podolin *et al.*[64] in high sucrose diet-fed rats where marine *n*-3 were able to prevent IR but not to reverse it once installed.

In summary, marine *n*-3 have no insulin-sensitising effect on patients with T2D.

Meta-analysis evaluating the effect of marine n-*3 on insulin sensitivity*

Several meta-analyses have assessed the effect of marine *n*-3 on insulin sensitivity. One difficulty is that many of them gather studies including patients with T2D and subjects with no diabetes. In addition, the dose and duration, the placebo used and the methods used to assess insulin sensitivity in studies included in these meta-analyses differ greatly, which contributes to possible if not probable confusing effects.

Akinkuolie *et al.*[98], in 2011, included eleven RCT, with subjects diabetic or non-diabetic, a dose of marine *n*-3 of 0·138 to 4 g/d except for one study with fatty fish, duration 8–16 weeks. The analysis used standardised mean difference (SMD) because of the different techniques used for assessing insulin sensitivity. They found no effect on insulin sensitivity in pooled analysis, but in the subgroup of evaluation by HOMA-IR, insulin sensitivity was significantly increased (SMD 0·30, 95% CI 0·03, 0·058). Gao *et al.*[99], in 2017, included seventeen RCT with 672 participants (healthy or T2D or obese or elderly). The duration was 4–24 weeks and the dose was EPA + DHA 1–4 g/d. They found no effect of *n*-3 in pooled analysis, but an improvement of insulin sensitivity in subjects with at least one symptom of metabolic disorders (five studies) (SMD 0·53, 95% CI 0·17, 0·88); $P < 0·001$). Abbott *et al.*[100], in 2016, included twenty-six RCT (fifteen RCT studied non-diabetic non-insulin-resistant subjects; ten included patients with T2D and one with a population of subjects with metabolic disorders). Two RCT evaluated the effect of 18:3 *n*-3 fatty acid and all others the effects of marine *n*-3. In the standardised pooled analysis (SMD), no effect of marine *n*-3 was observed. In trials of duration ≥6 weeks, a significant improvement in IR was seen in women ($P = 0·045$) but not in men ($P =$

https://doi.org/10.1017/S0029665120000087 Published online by Cambridge University Press

0·313). Brown *et al.*[101] included fourteen RCT using HOMA-IR as assessment of insulin sensitivity. No effect of marine *n*-3 was observed; a suspicion of a deleterious effect was reported for a dose of >4·4 g/d.

In summary, when considering these meta-analyses altogether, we can reasonably conclude that marine *n*-3 are able to increase insulin sensitivity. However, because some meta-analysis include RCT performed in very different groups of subjects, normal or obese or diabetic, the results may appear inconsistent when looking only at pooled analysis because of the great heterogeneity between RCT included. Thus, ideally considered should only be meta-analyses including RCT performed in homogeneous groups of subjects or those analysing separately the groups of RCT performed in similar groups of subjects (healthy or diabetic or obese non-diabetic). As summarised earlier, including a meta-analysis RCT performed in patients with T2D will certainly mask an insulin-sensitising effect because marine *n*-3 have no effect on patients with marked (severe) IR.

Other meta-analyses aimed to evaluate the effects of marine *n*-3 on insulin sensitivity during gestational diabetes, polycystic ovary syndrome and non-alcoholic fatty liver disease. Zhong and Wang[102] included five RCT assessing the effects of marine *n*-3 in patients with gestational diabetes. They concluded an improvement in fasting blood glucose ($P = 0·003$) and HOMA-IR ($P = 0·002$). Sadeghi *et al.*[103], in 2016, included three RCT involving seventy-two cases of patients with ovary polycystic syndrome and seventy-three controls. The dose was 1·2–3·6 g/d over 6–8 weeks. No effect on insulin sensitivity was observed. Yang *et al.*[104], in 2018, included nine RCT (591 patients with ovary polycystic syndrome); marine *n*-3 (900–4000 mg/d; duration 6–12 weeks) improved HOMA-IR ($P < 0·00001$) and increased plasma adiponectin ($P = 0·002$). This meta-analysis is in accordance with the conclusion of the previous one. Concerning adiponectin, it also confirms the meta-analysis of fourteen RCT by Wu *et al.*[105], in 2013, concluding a significant increase in plasma adiponectin ($P = 0·02$) following marine *n*-3 supplementation. Yan *et al.*[106], in 2018, included eighteen RCT evaluating the effect of *n*-3 on insulin sensitivity in patients with non-alcoholic fatty liver disease (0·25–5 g/d, duration 3–18 months). They observed a significant improvement of HOMA-IR and glycaemia but not of insulinaemia.

In summary, these meta-analyses consistently conclude that there is an improvement in HOMA-IR and an increase in plasma adiponectin in patients with gestational diabetes, polycystic ovary syndrome or non-alcoholic fatty liver disease, suggesting an insulin-sensitising effect of marine *n*-3 when IR is probably moderate.

Meta-analysis evaluating the effect of marine n-*3 on the risk of developing type-2 diabetes*

Zheng *et al.*[107], in 2012, included twenty-four studies (24 509 T2D patients and 545 275 participants). In the cohorts studies, there was no association between marine *n*-3 and the risk of T2D (relative risk (RR) 1·07 (95 % CI: 0·95, 1·20)). Subgroups analysis indicated for Asian population RR 0·87 (95 % CI 0·79, 0·96) and 1·16 (95 % CI 1·04, 1·28) for Western populations. There was no significant heterogeneity for Asian studies while it was high for US studies.

Zhou *et al.*[108], in 2012, included seven publications (ten cohorts; 506 665 participants). Comparing the highest *v.* the lowest intake of *n*-3, the pooled relative risk was 1·076 (non-significant) with a high between-study heterogeneity.

Wu *et al.*[109], in 2012, found no association with the risk of T2D in a pooled analysis of cohorts and the consumption of EPA + DHA (*n* 16 cohorts; RR per 250 mg/d = 1·04) or circulating levels of EPA/DHA biomarkers (*n* 5 cohorts; RR per 3 % of total fatty acids 0·94). Because of a high heterogeneity, an analysis in subgroups was performed and concluded that in studies in Asia, EPA/DHA consumption was associated with lower incidence of T2D (per 250 mg/d, RR 0·95, 95 % CI 0·91, 0·99), with minimal heterogeneity across studies, whereas in studies in North America/Europe, EPA/DHA consumption was associated with higher incidence of T2D (per 250 mg/d, RR 1·12, 95 % CI 1·05, 1·20), with substantial remaining heterogeneity. The same result was observed when considering fish/seafood, which was associated with lower T2D risk in Asia cohorts (RR per 100 g/d = 0·89, 95 % CI 0·81, 0·98), and higher risk in North America/Europe cohorts (RR per 100 g/d = 1·38, 95 % CI 1·13, 1·70).

Wallin *et al.*[110], in 2012, included sixteen studies involving 527 441 participants and 24 082 diabetes cases. There was a high heterogeneity between studies. For each 0·30 g daily increment in dietary marine *n*-3 intake the corresponding RR for USA, Europe and Asia/Australia were 1·17 (95% CI 1·09, 1·26), 0·98 (95% CI 0·70, 1·37) and 0·90 (95% CI 0·82, 0·98).

Muley *et al.*[111], in 2014, analysed the pooled effect of seven times increase in fatty fish intake. They observed a decrease in the risk of T2D (RR 0·89; $P = 0·028$). To decrease the high heterogeneity of the studies, the authors formed subgroups by region. As in Chen's meta-analysis, this brought the heterogeneity in Asian at minimal but the heterogeneity remained high in USA studies. The subgroup analysis showed a constant reduced risk of T2D in Asian with three, six or ten times increase in fatty fish (RR 0·90) while the risk proportionally increased in US population (RR 1·078, 1·162 and 1·24, respectively).

Chen *et al.*[112], in 2017, included five articles with ten cohort trials (426 852 participants) with a follow-up of 4·1–18 years. The effect of EPA or DHA alone and of EPA + DHA was analysed. There was no significant association between overall marine *n*-3 intake and the risk of T2D (RR 1·14, $P = 0·062$), as well as between EPA + DHA and risk (RR 1·07, $P = 0·35$). However, considering EPA or DHA alone the risk was increased (RR 1·45, $P < 0·001$). The analysis identified a non-linear association describing an inverted U-shape curve with 0·43 g/d *n*-3 as the peak point. Because of a high heterogeneity in the overall analysis, the authors performed a

https://doi.org/10.1017/S0029665120000087 Published online by Cambridge University Press

subgroup analysis by ethnicity (Asians *v.* Westerners), study duration and age of participants at recruitment. Studies based on Asian population showed a protective effect of marine *n*-3 to the risk of T2D (RR 0·82, $P < 0·001$) and an increased risk in Westerners (RR 1·30, $P < 0·001$). It is to note that there was a high degree of heterogeneity in studies on Westerners while there was no heterogeneity in studies in Asian. A duration of the studies ≥16 years was associated with an increased risk (RR 1·33, $P < 0·001$) as well as an age >54 years at inclusion (RR 1·24, $P = 0·04$). There was no association for duration <16 years and an age <54 years at inclusion.

Summarising these meta-analyses, we can conclude, when pooled analysis of cohorts is performed, a lack of association between marine *n*-3 intake and the risk of T2D, knowing that in all meta-analyses a high heterogeneity between studies was found. The analysis by geographical subgroups showed that marine *n*-3 decreased the risk of T2D in Asian while increasing it in Westerners. This should be taken with caution inasmuch as the subgroup analysis almost suppressed the heterogeneity in Asian studies whereas it remained high in Western studies. So, if it is very probable that marine *n*-3 decrease the risk of T2D in Asian, it remains to be proved that they increase it in Westerners. This has been well discussed by Rice Bradley in his recent review[113].

Forouhi *et al.*[114], in 2016, measured plasma phospholipid PUFA among 12 132 incident T2D cases and 15 919 subcohort participants in the European Prospective Investigation into Cancer and Nutrition-InterAct study across eight European countries, with a follow-up of 10 years. They found no association between EPA and DHA and the risk of T2D.

In the Finnish Diabetes Prevention Study[115], including overweight middle-aged patients followed over 11 years, the baseline proportions of EPA and DHA were associated with a 25 % ($P \leq 0·01$) lower incidence of T2D during a median follow-up of 11 years.

Thus, due to these contrasted if not contradictory results, it is difficult to conclude in Western populations a protective, a neutral or a deleterious effect of marine *n*-3.

Conclusion

The response to the title of our paper is yes, marine *n*-3 are protective towards IR, but not in all people and not at low daily dose.

At biochemical levels, they minimise or abolish several alterations leading to IR, by modulating transcription factors involved in insulin signalling, inflammation, lipogenesis, fatty acid oxidation and other pathways. In rodent models of dietary-induced IR, they clearly prevent IR. However, these protective effects have generally been observed with a very high dose not extrapolable to the amount which can be consumed or taken by human subjects.

Epidemiological studies in population consuming high doses of marine *n*-3 (Inuits) have shown a very low incidence of T2D when traditional diet was consumed; this incidence increased following substitution of a western diet for the traditional diet. Intervention studies aiming to reintroduce in Inuits their traditional diet showed a decrease in incidence of T2D.

Several studies and meta-analyses have confirmed in healthy subjects and in patients with illnesses characterised by moderate IR (gestational diabetes, polycystic ovary syndrome, non-alcoholic fatty liver disease and metabolic syndrome) their ability to increase insulin sensitivity at a dose of at least 1·8 g/d EPA + DHA. Conversely, they have no effect, whatever the dose given, on IR of patients with T2D.

Their ability to prevent T2D in subjects at risk remains debated. Several meta-analyses concluded a protective effect on Asian and an increased risk on Westerners, but heterogeneity of studies included was low in Asian populations and high in western population, which may explain the result in Westerners.

Marine *n*-3 must not be associated with glucocorticoids because they further aggravate glucocorticoid-induced IR.

Very probably, the sooner and the longer they are consumed throughout the lifecycle, the better they can be protective to IR, in association of course with the maintenance of a normal weight and regular physical exercise and/or by fighting against sedentary lifestyles. The daily amount of their beneficial effect, although not clearly defined is probably, in view of the studies available, somewhat higher than current recommendations. Ideally the amount should be of at least 1·8 g/d EPA + DHA, which could be prone in subjects at risk of T2D. Such an amount can be reached by the consumption of three portions of fatty fish weekly. For those not liking fish, vegetarians or with allergy to fish proteins, concentrated fish oils of pharmaceutical quality can be used.

Updating of specific recommendations of their usefulness for prevention of IR states by a panel of international independent experts would probably be useful.

Financial Support

None.

Conflict of Interest

None.

Authorship

The author had sole responsibility for all aspects of preparation of this paper.

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