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Session 3: Fatty acids and the immune system
Regulation of adipokine secretion by *n*-3 fatty acids

María J. Moreno-Aliaga*, Silvia Lorente-Cebrián and J. Alfredo Martínez
Department of Nutrition, Food Science, Physiology and Toxicology, University of Navarra, Pamplona, Spain

Obesity leads to several chronic morbidities including type 2 diabetes, dyslipidaemia, atherosclerosis and hypertension, which are major components of the metabolic syndrome. White adipose tissue (WAT) metabolism and WAT-derived factors (fatty acids and adipokines) play an important role in the development of these metabolic disturbances. In fact, dysregulated adipokine secretion from the expanded WAT of obese individuals contributes to the development of systemic low-grade inflammation, insulin resistance and metabolic syndrome. The *n*-3 PUFA EPA and DHA have been widely reported to have protective effects in a range of chronic inflammatory conditions including obesity. In fact, *n*-3 PUFA have been shown to ameliorate low-grade inflammation in adipose tissue associated with obesity and up-regulate mitochondrial biogenesis and induce beta-oxidation in WAT in mice. Moreover, the ability of *n*-3 PUFA to regulate adipokine gene expression and secretion has been observed both *in vitro* and *in vivo* in rodents and human subjects. The present article reviews: (1) the physiological role of adiponectin, leptin and pre-B cell colony-enhancer factor/visfatin, three adipokines with immunemodulatory properties involved in the regulation of metabolism and insulin sensitivity and (2) the actions of *n*-3 PUFA on these adipokines focusing on the underlying mechanisms and the potential relationship with the beneficial effects of these fatty acids on obesity-associated metabolic disorders. It can be concluded that the ability of *n*-3 PUFA to improve obesity and insulin resistance conditions partially results from the modulation of WAT metabolism and the secretion of bioactive adipokines including leptin, adiponectin and visfatin.

Leptin: Adiponectin: Visfatin: *n*-3 fatty acids: Obesity: Inflammation

Adipokines, obesity and inflammation

Obesity represents an increasing problem of health care. It leads to several chronic morbidities including type 2 diabetes, dyslipidaemia, atherosclerosis and hypertension, which are major components of the metabolic syndrome[1]. Obesity also predisposes individuals to an increased risk of developing non-alcoholic fatty liver disease and certain cancers[2]. Furthermore, obesity and impaired immune function have been described in both human subjects and genetically obese rodents, supporting a link between adipose tissues and immunocompetent cells[3].

Adipose tissues play crucial roles in the development of obesity, with white adipose tissue (WAT) functioning as an energy storage organ and brown adipose tissue as an energy consumption organ. Several studies have suggested the importance of WAT metabolism and WAT-derived factors (fatty acids and adipokines) in the development of obesity and systemic insulin resistance, the key event in the pathophysiology of the metabolic syndrome[1].

WAT is an important secretory organ which produces a number of molecules that putatively play critical roles in fuel homeostasis and contribute to maintain metabolic control. These bioactive molecules, generally termed 'adipokines', include leptin, adiponectin, TNFα, IL-6, omentin, visfatin and apelin, among others[4]. In fact, the number of adipokines has enlarged considerably during the last few years and nowadays more than 50 have been identified[5]. These adipokines are involved in the physiological regulation of fat storage, adipogenesis, energy metabolism, food

Abbreviations: AMPK, AMP-activated protein kinase; WAT, white adipose tissue.

***Corresponding author:** Dr María J. Moreno-Aliaga, fax + 34 948 42 56 49, email mjmoreno@unav.es

intake and also play an important role in metabolic disorders. Adipokines may exert their physiological functions in WAT locally (autocrine/paracrine) and systemically (endocrine) and, in addition, WAT also expresses a high number of important receptors leading to the interaction between different organs and tissues involved in energy homeostasis, such as central nervous system, liver, skeletal muscle and pancreas[5]. Thus, WAT participates in a wide range of biological processes including the regulation of energy metabolism and insulin sensitivity.

Indeed, the development of obesity and accompanying comorbidities is associated with an altered function of the adipocytes, especially concerning the synthesis and secretion of adipokines. Thus, obesity is frequently associated with increased levels of leptin and apelin, while adiponectin and omentin concentrations are decreased, being closely related to insulin resistance[6–8]. Moreover, hypertrophic WAT secretes various pro-inflammatory adipocytokines including TNFα and IL-6[9]. In fact, obesity has been associated with systemic low-grade inflammation, which has been suggested to play an important role in the pathogenesis of obesity-related disorders, such as insulin resistance and atherosclerosis[10].

In this review, we provide an overview of recent advances in the understanding of adipose tissue and its secreted bioactive peptides termed 'adipokines' focusing on adiponectin, leptin and pre-B cell colony-enhancer factor/visfatin, three adipokines with immune modulatory properties that directly regulate metabolism and insulin sensitivity and their regulation takes place by *n*-3 PUFA.

n-3 PUFA in the prevention of obesity and linked disorders

A growing body of evidence suggests that the amount and type of fat[11,12] included in the diet contribute to the development of obesity and insulin resistance. Specifically, diets high in saturated fat promote obesity and insulin resistance in rodents[13], whereas diets high in fish oil appear to prevent or attenuate the development of these diseases[14]. In fact, there are many evidences suggesting that the intake of *n*-3 PUFA, namely DHA (22:6*n*-3) and EPA (20:5*n*-3), produce some benefits on CVD markers, insulin resistance, obesity and serum lipids both in rodents[15–19] and human subjects[20]. During the last few years, research focused on the study of the mechanisms underlying the beneficial effects of marine *n*-3 PUFA consumption. Several studies have indicated that *n*-3 PUFA exert their hypolipidaemic and anti-obesity effects by co-ordinately suppressing new fatty acid synthesis and by inducing fatty acid oxidation in different tissues, such as liver, skeletal muscle and WAT[16,19]. Furthermore, it was demonstrated that *n*-3 PUFA are important regulators of gene expression acting through the PPAR and sterol regulatory element-binding protein pathways[21,22], which are two critical transcriptional factors involved in β-oxidation (PPARα) and lipogenesis, respectively.

Moreover, it has been suggested that *n*-3 PUFA could prevent insulin resistance linked to obesity by the prevention of the decreased expression of GLUT4 in both skeletal muscle and adipose tissue[23,24] and by regulating 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[23].

Furthermore, *n*-3 PUFA have been widely reported to have anti-inflammatory effects in a range of chronic inflammatory conditions including rheumatoid arthritis and Crohn's disease[25]. Treatment of obese subjects with *n*-3 PUFA in a clinical setting has been shown to reduce circulating levels of both pro-inflammatory cytokines and acute phase proteins[26]. The beneficial actions of *n*-3 PUFA were initially believed to be mediated by a decrease in the production of classic inflammatory mediators, such as arachidonic acid-derived eicosanoids and inflammatory cytokines[27]. However, in recent years, *n*-3 PUFA have been demonstrated to serve as substrates for the conversion to a novel series of lipid mediators designated resolvins and protectins[28] which have been proposed to mediate between the protective and beneficial actions underlying the effects of *n*-3 PUFA[29]. The role of these lipid mediators in the beneficial effects of *n*-3 PUFA in obesity and its linked disorders has been demonstrated in a recent study in *ob/ob* mice, showing that increased intake of *n*-3 PUFA not only inhibited the formation of eicosanoids derived from the *n*-6 PUFA arachidonic acid, but also increased the generation of protective *n*-3 PUFA-derived lipid mediators (protectins and resolvins), which mimicked the insulin-sensitizing and antisteatotic effects exerted by *n*-3 PUFA. Interestingly, the effects of resolvins and protectins appeared to be more potent than their *n*-3 precursors[30].

Furthermore, several recent studies have suggested that the improvements in lipid metabolism and prevention of obesity and diabetes described for *n*-3 PUFA partially result from the modulation of metabolism and secretory functions of adipose tissue[31]. In fact, *n*-3 PUFA have been shown to ameliorate low-grade inflammation in adipose tissue associated with obesity[32,33] and up-regulate mitochondrial biogenesis and induce beta-oxidation in WAT in mice[16]. Moreover, the ability of *n*-3 PUFA to regulate adipokine gene expression and secretion has been observed both *in vitro* and *in vivo* in rodents[17,34] and human subjects[35].

Leptin

Leptin is a hormone primarily secreted by WAT, although it is also produced by several other tissues, such as placenta, stomach, bone marrow and brain[36]. Leptin is involved in the regulation of food intake, energy expenditure, body fat storage and insulin signalling[6]. In leptin-sensitive subjects, short-term increases in circulating leptin in response to feeding promote satiety. The key role of the leptin system in regulating body fat in animals and human subjects is demonstrated by severe hyperfagia and obesity caused by leptin deficiency[37,38]. However, circulating leptin concentrations are correlated with adiposity in human subjects[39] and animals[40], suggesting that resistance to leptin action develops with chronic overfeeding and obesity[41]. Increased sensations of hunger during dieting are related to the magnitude of decreases in leptin[42]. In addition, it was

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demonstrated that the normal compensatory decreases of energy expenditure and thyroid axis function in response to energy-restricted diet in human subjects were prevented by low-dose leptin replacement. Thus, although leptin administration may not promote substantial weight loss in obese leptin-resistant subjects, leptin therapy may be useful to maintain the weight loss achieved by dieting[43].

Leptin also participates in the regulation of the activity of the reproductive[44] and immune system[45]. In fact, leptin administration corrects many of the neuroendocrine, reproductive, metabolic and immune system deficits associated with leptin deficiency[46]. With regard to the role of leptin in inflammation and immunity, the expression of leptin is increased in conditions that are associated with the release of pro-inflammatory cytokines, such as acute inflammatory conditions during sepsis[47]. In human adipose tissue, TNFα and IL-6 increases leptin mRNA only when added together with dexamethasone. It has been suggested that the increase in local cortisol and inflammatory cytokines in adipose tissue may contribute to higher leptin mRNA levels in obese subjects and to higher leptin levels observed after endotoxin administration[41].

Congenital deficiency of leptin has been associated with increased frequency of infection and related mortality and it was hypothesized that a low concentration of serum leptin might contribute to increased susceptibility to infection[45]. Several studies have investigated the effects of leptin on innate and adaptive immune responses[48]. In innate immunity, the ability of leptin to up-regulate the phagocytic function of macrophages/monocytes in mice and human subjects has been described. In macrophages, leptin increases the secretion of pro-inflammatory cytokines, such as TNFα (early), IL-6 (late) and IL-12[49,50]. Furthermore, leptin stimulates neutrophil chemotaxis and the production of reactive oxygen species by these cells and regulates natural killer-cell differentiation, proliferation, activation and cytotoxicity[51,52]. The most evident effects of leptin on the modulation of adaptive immune responses have been shown in leptin-deficient mice (*ob/ob*) and human subjects, which exhibited immune abnormalities, such as thymic hypotrophy and reduced secretion of inflammatory cytokines, in parallel with metabolic disturbances[53]. These alterations are reversed by the administration of recombinant leptin[46].

Furthermore, patients with autoimmune diseases have shown high serum levels of leptin, suggesting that this adipokine might be either a contributing factor or a marker of disease activity. It has also been observed that leptin administration accelerates autoimmune diabetes in female non-obese diabetic mice[48]. In summary, leptin has pro-inflammatory effects. This could be detrimental in many animal models of inflammatory and autoimmune disease, but might be protective in several infectious disease settings[54].

Regulation of leptin by n-3 *PUFA*

Several studies from our group and others have demonstrated the ability of dietary *n*-3 PUFA to modulate leptin gene expression and secretion both *in vitro* and *in vivo*. Thus, *in vitro* studies with EPA (0·1–200 μl) showed the ability of this fatty acid to stimulate in a dose-dependent manner leptin mRNA expression and leptin secretion in 3T3-L1 cells[55] and in primary rat adipocytes[34]. Little is still known about the mechanisms underlying this stimulatory action of EPA on leptin. Insulin-stimulated glucose metabolism has been described as a major determinant of leptin production[56–58]. EPA increases glucose utilization, decreases anaerobic metabolism of glucose to lactate and increases glucose oxidation. Moreover, the ability of EPA to increase leptin production was found to be highly correlated with its effects to decrease anaerobic glucose metabolism to lactate[34]. However, Cammisotto *et al.*[59] reported an inhibitory effect of EPA (1 mM) on insulin-stimulated leptin secretion in white adipocytes. The disparity may be related to differences in the duration of the cultures (short *v.* long), the type of culture system employed and the higher concentration of insulin and EPA used in this study.

Several *in vivo* studies in rats and mice have reported that prolonged intake of diets high in *n*-3 PUFA resulted in significant decreases in plasma leptin[60,61], which are likely to be secondary to decreases observed in WAT mass. However, a study of our group observed that the administration of highly purified EPA (1 g/kg) during 35 d significantly decreased the leptin circulating levels in lean rats fed on a standard diet, while a significant increase of the adipokine was observed in overweight rats treated with fatty acid. Similarly, leptin gene expression in epididymal fat showed the same pattern as circulating levels[17]. This is in agreement with the study of Peyron-Caso *et al.*[62], which also described an increase in leptin concentrations in rats fed an *n*-3 PUFA-enriched diet. In addition, Rossi *et al.*[63] observed that dietary fish oil positively regulates the plasma leptin levels in sucrose-fed, insulin-resistant rats. Taken together, these data suggest that *n*-3 PUFA actions on leptin seem to be dependent on diet composition and the physiological and metabolical status of animals, which could be important to take into account when considering supplementation with *n*-3-enriched products. Studies about the effects of *n*-3 PUFA on leptin levels in human subjects have shown that the inclusion of either lean (150 g cod, three times per week) or fatty fish (150 g salmon, three times daily), or six fish oil capsules (approximately 3 g/d containing EPA + DHA) as part of an energy-restricted diet resulted in approximately 1 kg more weight loss, which was accompanied by a decrease in fasting insulin and leptin levels[64].

Adiponectin

Adiponectin is a hormone mainly secreted by adipose tissue and to a small degree is also produced by cardiac myocytes, muscle cells and endothelial cells with important metabolic effects[6]. Adiponectin exists both as a full-length protein, and a proteolytic cleavage fragment (globular adiponectin). Full-length adiponectin is a trimer (low molecular weight adiponectin) that forms hexamers (medium molecular weight adiponectin), which can further oligomerize to form polymers (high molecular weight form). Bioactivity studies have suggested that high molecular weight rather than low

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molecular weight are the functional components of adiponectin as it relates to the regulation of insulin sensitivity[65]. Plasma adiponectin concentrations are decreased in obesity and weight loss leads to an increase in the adiponectin circulating level[66]. Furthermore, intracerebroventricular administration of adiponectin decreased body weight mainly by stimulating energy expenditure, without affecting food intake in mice[67]. Circulating levels of adiponectin have been positively associated with whole-body insulin sensitivity[68]. In fact, low adiponectin levels have been shown to precede and predict the onset of insulin resistance, type 2 diabetes and CVD[68,69]. In this context, it has been observed that adiponectin *knockout* mice are more sensitive to diet-induced insulin resistance[70,71]. On the contrary, increasing endogenous adiponectin levels lead to improved insulin sensitivity[72] as it reverses the insulin resistance associated with both lipoatrophy and obesity[68]. In this context, it has also been observed that treatment with thiazolidinedione drugs, which are insulin sensitizers, increases the plasma adiponectin levels[73]. Moreover, it has been suggested that adiponectin may mediate some cardioprotective and insulin-sensitizing effects through its anti-inflammatory properties. Thus, adiponectin has been shown to inhibit endothelial NF-κB signalling[74] and markedly reduce the phagocytic activity and TNFα production in cultured macrophages[75]. Adiponectin also induces the production of anti-inflammatory cytokines IL-10 and IL-1RA in human leucocytes[76]. Moreover, adiponectin-deficient mice have increased the levels of TNFα mRNA in adipose tissue and higher TNFα circulating levels[71]. In addition, pro-inflammatory cytokines, such as TNFα and IL-6, inhibit adiponectin gene expression and secretion in adipocytes[77], suggesting that the pro-inflammatory state associated with obesity could contribute to the decreased levels of adiponectin observed in obese subjects. In turn, this lower synthesis of adiponectin might lead to dysregulation of the controls that inhibit the production of pro-inflammatory cytokines, resulting in an overwhelmingly pro-inflammatory state[54].

Regulation of adiponectin by n-3 *PUFA*

During the last few years, several studies suggested that the insulin-sensitizing properties of dietary fish oils could be related to the increase in circulating levels of adiponectin both in rodents and human subjects. The study of Flachs *et al.*[78] observed that feeding mice with a high-fat diet enriched with EPA/DHA concentrate (6% EPA, 51% DHA) for 5 weeks leads to elevated systemic concentrations of adiponectin and suggested that this increase could explain, to some extent, the anti-diabetic properties of these *n*-3 PUFA. Rossi *et al.*[63] also found that dietary fish oil positively regulates the plasma adiponectin levels in sucrose-fed, insulin-resistant rats. A recent study by Gonzalez-Periz *et al.*[30] reported increased adipose adiponectin mRNA levels in *ob/ob* mice receiving a diet enriched with *n*-3 PUFA for 5 weeks. Moreover, a study of our group demonstrated that the ability of adipocytes to produce adiponectin was significantly increased by the administration of highly purified EPA ethyl ester in both lean and high-fat-induced overweight rats. These changes in adiponectin were inversely related to the homeostatic model assessment index, a marker of insulin resistance, suggesting that the EPA-stimulation of adiponectin could contribute to the insulin-sensitizing properties of this *n*-3 fatty acid[34]. Furthermore, a 3-month treatment with EPA (1·8 g daily) in human obese subjects has also been shown to increase adiponectin secretion[35].

Regarding the mechanisms involved in the stimulatory action of *n*-3 PUFA on adiponectin, Neschen *et al.*[79] found that the up-regulation of adiponectin secretion by fish oil *in vivo* is mediated by a PPARγ-dependent and PPARα-independent manner in mice epididymal fat. However, these authors also found that in clear contrast to what was observed after *in vivo* administration, *n*-3 fatty acids failed to stimulate adiponectin mRNA expression in 3T3-L1 adipocytes. Moreover, we found that long-term exposure of primary cultured adipocytes to EPA (200 μM) significantly decreased adiponectin gene expression and protein secretion and reduced PPARγ mRNA levels, suggesting that the inhibition of adiponectin by EPA is likely to be secondary to the down-regulation of this transcription factor[80]. These features suggest that the stimulation of adiponectin secretion observed after *n*-3 PUFA administration to rodents and human subjects involves an indirect mechanism or that they require *in vivo* metabolic processing to do so[79].

In addition, using an *in vitro* co-culture of adipocytes and macrophages, Itoh *et al.*[35] showed that EPA (50–200 μM) reversed the co-culture-induced decrease in adiponectin secretion at least in part through down-regulation of TNFα in macrophages. These investigators suggested that *in vivo* EPA could increase adiponectin secretion at least partly by interrupting the vicious cycle created by adipocytes and macrophages in human obese subjects as in the *in vitro* co-culture experiments.

AMP-activated protein kinase (AMPK) is a fuel-sensing enzyme that acts as a gatekeeper of energy balance by regulating glucose and lipid homeostasis in adipose, liver and muscle tissues[81]. Furthermore, the anti-diabetic efficacy of some insulin sensitizers, such as metformin and glitazones, involves the activation of AMPK[82]. AMPK activation has been shown to stimulate the production of adiponectin by adipocytes[83]. It has been suggested that AMPK activation could be involved in *n*-3 PUFA-induced improvements on insulin sensitivity[31]. A recent study of our group has demonstrated that EPA (100–200 μM) strongly stimulates AMPK phosphorylation in 3T3-L1 adipocytes[84]. Moreover, two recent trials have described the ability of *n*-3 PUFA to activate AMPK *in vivo*[30,85]. Thus, Kopecky *et al.*[85] showed that both α1 AMPK and phosphorylated AMPK contents increase significantly in mice fed a high-fat diet with 44% of its lipid replaced by *n*-3 long-chain PUFA concentrate EPAX 1050 TG for 5 weeks. Gonzalez-Periz *et al.*[30] observed that the AMPK activity was significantly increased in *ob/ob* mice receiving DHA at a dose of 4 μg/g body weight every 12 h for 4 d. These observations have led to suggest that AMPK activation could be a potential mechanism underlying the stimulatory effects of *n*-3 PUFA on adiponectin levels. Future studies are necessary to better characterize the mechanism involved in the *n*-3 PUFA stimulatory effect on adiponectin *in vivo*.

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Pre-B cell colony-enhancer factor/visfatin

Visfatin was identified as an adipokine that was highly expressed in visceral fat of human subjects and rodents, whose plasma circulating levels were positively correlated with the size of visceral fat depots[86]. Besides, this adipokine seemed not to be correlated with subcutaneous fat depots and consequently, it was termed *visfatin* (from visceral fat). This adipocytokine was firstly isolated more than 10 years ago from lympocytes as a 52-kDa protein called pre-B cell colony-enhancer factor[87], because it favoured the development of lymphocyte B colonies, inhibited apoptosis in neutrophils and was also linked to several inflammatory diseases[88]. Moreover, this adipokine, which has also been named nicotinamide phosphoribosyltranferase, was found to catalyse the first step in NAD biosynthesis from nicotinamide[89]. In addition to visceral fat, visfatin/pre-B cell colony-enhancer factor/nicotinamide phosphoribosyltranferase is also expressed in bone marrow, liver, muscle and kidney among other tissues, where it is involved in a wide variety of functions including reproduction and immunity[90].

Visfatin and inflammation

Several studies have demonstrated that visfatin induces pro-inflammatory cytokine production, such as TNFα, IL-1β, IL-6, in CD14+ monocytes[88,91]. When administered to mice, the murine pre-B cell colony-enhancer factor/visfatin significantly increased the level of circulating IL-6[91]. Moreover, visfatin promotes macrophage survival, which could affect the balance of macrophage survival and death in the setting of obesity, which in turn could play important roles in obesity-associated diseases[92]. Several studies now support the evidence that visfatin is primarily a pro-inflammatory cytokine, as its serum/plasma levels are increased in various inflammatory disorders[88]. Thus, recent research has shown that visfatin may be a direct contributor to vascular inflammation[93] and that plasma visfatin levels are significantly higher in chronic coronary artery diseases and acute coronary syndromes[94]. However, the role played by TNFα, IL-6 and other pro-inflammatory cytokines in visfatin production by adipocytes is still controversial. Indeed, while some studies have observed that TNFα and IL-6 inhibit visfatin synthesis in 3T3-L1 adipocytes[95,96], an assay performed in human adipocytes has shown that treatment with TNFα induces an up-regulation in visfatin production[97].

Role of visfatin on obesity and insulin resistance

The role of visfatin in obesity and linked metabolic disorders remains unclear. Thus, in KKAy mice, an experimental model for obesity and type 2 diabetes, plasma visfatin was significantly increased during obesity development. This was also accompanied by the enhancement of visfatin mRNA expression in visceral adipose tissue, but not in the liver and subcutaneous adipose tissue. Moreover, it was also described that mice fed a high-fat diet showed increased plasma visfatin concentrations, which were also associated with increases of visfatin mRNA levels in visceral mesenteric fat[86]. However, Kloting *et al.*[98] reported no significant change in relative visfatin gene expression in adipocytes of subcutaneous and epididymal fat depots in Wistar–Ottawa–Karlsburg W rats, a model of polygenic metabolic syndrome.

Heterozygous visfatin$^{+/-}$ mice were not obese and their insulin circulating levels were not significantly different to wild-type mice. Glucose plasma levels were higher in visfatin$^{+/-}$ mice in fasting and after re-feeding, but glucose tolerance tests did not show any significant difference between both groups.

On the other hand, several studies have suggested that up-regulation of visfatin could be a potential mechanism mediating between the effects of PPAR (PPARα, PPARγ and PPARδ) agonists on insulin sensitivity in Otsuga Long–Evans Tokushima fatty rats and Wistar rats fed a high-fat diet[99,100].

The role of visfatin in human obesity is controversial. While some studies have observed a positive correlation between visfatin plasma levels and visceral obesity[86,101,102], others have argued against these data. In fact, Berndt *et al.*[103] did not find any correlation between visfatin plasma levels and visceral fat mass in obese subjects. Hammarstedt *et al.*[104] also reported no association between circulating visfatin levels and fat abdominal content and/or with waist:hip ratio in people with diabetes. On the other hand, other studies have found an inverse relationship between visfatin and obesity development. A recent study showed that plasma visfatin was negatively correlated with visceral fat in human subjects genetically predisposed to obesity[105]. Besides, they associated visfatin circulating levels with a beneficial lipid profile in non-diabetic subjects. Moreover, Pagano *et al.*[106] reported that while visfatin mRNA expression in visceral adipose tissue was enhanced in obesity, visfatin circulating and gene expression levels in subcutaneous adipose tissue were significantly decreased in obese subjects.

Further studies related to weight loss in obese subjects with changes in circulating visfatin levels have not provided concluding evidence. Thus, while some trials described enhancements on plasma visfatin levels after a decrease of fat depots and weight loss[107,108], others found a significant reduction in visfatin levels after massive weight loss following bariatric surgery[109,110].

Several human studies investigating visfatin and insulin-resistance have demonstrated a positive correlation between increased visfatin concentrations with type 2 diabetic and obese subjects[111,112]. However, others did not find any correlation between visfatin plasma levels and several parameters of insulin resistance, such as the homeostatic model assessment index, suggesting that visfatin is not related to insulin resistance in human subjects[103,106]. In this context, and contrary to observations in rodents, Hammarstedt *et al.*[104] reported that neither gene expression nor visfatin circulating levels are regulated by thiazolidinediones in human subjects, suggesting that visfatin is not involved in the insulin-sensitizing properties of these drugs.

Another conflicting point regarding visfatin actions is the potential insulin-mimetic properties of this adipokine initially described by Fukuhara *et al.*[86]. However, these actions of visfatin are in doubt, because such findings have not been corroborated by other investigators[89,113].

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Regulation of visfatin by n-3 *PUFA*

A previous study by our group showed that the oral supplementation of EPA ethyl ester (1 g/kg) during 35 d was able to prevent the decrease of visfatin gene expression observed in high fat diet-induced obese rats. Moreover, we found an inverse relationship with the homeostatic model assessment index, suggesting that the insulin-sensitizing effects of EPA could be related to its stimulatory action on visfatin gene expression in visceral fat[18]. In a recent trial, we demonstrated a direct stimulatory effect of EPA (200 μM) on both visfatin gene expression and protein secretion in primary rat and 3T3-L1 adipocytes, suggesting that the up-regulation of visfatin gene expression in visceral adipose tissue observed after *in vivo* EPA administration was not only due to the reducing effects of EPA treatment on the size of this fat depot, but also by a direct transcriptional up-regulation of visfatin gene by this *n*-3 PUFA[84].

Other studies have reported the ability of dietary fatty acids to modulate visfatin gene expression. In contrast to EPA, palmitate and oleate (0·125–1 mM) down-regulated visfatin mRNA gene expression in 3T3-L1 adipocytes[114]. Moreover, this down-regulation of visfatin was mentioned as a potential mechanism to directly induce insulin resistance by oleate and palmitate *in vitro*[114]. In this context, it has also been shown that a synthetic mixture including stearic, oleic, linoleic, linolenic and arachidonic acid normalized the increase in visfatin release induced by treatment with the insulin-sensitizing PPARγ agonist rosiglitazone in human-isolated adipocytes[115]. These findings suggest a differential regulation of visfatin depending on the type of dietary fat and support our hypothesis that visfatin up-regulation by EPA could be another mechanism by which *n*-3 PUFA may improve insulin sensitivity. An interesting finding of our study was that the stimulatory effect of EPA on visfatin secretion in adipocytes involved the AMPK activation pathway[84].

Conclusion

Low-grade inflammation has been identified as a key factor in the development of metabolic syndrome features affecting obese subjects, leading to type 2 diabetes and CVD. In obesity, the expanding adipose tissue makes a substantial contribution to the development of obesity-linked inflammation via dysregulated secretion of pro-inflammatory cytokines, chemokines and adipokines and the reduction of anti-inflammatory adipokines, such as adiponectin. In this context, *n*-3 PUFA have been shown to prevent and/or ameliorate inflammation in key metabolic organs including adipose tissue, liver and muscle. Indeed, the *n*-3 PUFA EPA and DHA have been widely reported to have protective effects in a range of chronic inflammatory conditions including obesity, insulin resistance and CVD. From the present review, it can be concluded that these beneficial properties of *n*-3 PUFA partially result from the modulation of WAT metabolism and the secretion of bioactive adipokines (such as leptin, adiponectin and visfatin) that directly regulate nutrient metabolism and insulin sensitivity. Taking into account the beneficial actions of *n*-3 PUFA, several government and health organizations worldwide have promoted *n*-3 PUFA consumption, and in general, recommendations for prevention are lower than for treatment of diseases. High quality and purity *n*-3 PUFA supplements have been proposed to get therapeutically significant doses in patients with different pathologies. However, questions have been raised about the recommendation of eat more fish or take fish oil supplements as a source of *n*-3 PUFA for diseases prevention[116–120].

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