

# (n-3) Fatty Acids Alleviate Adipose Tissue Inflammation and Insulin Resistance: Mechanistic Insights<sup>1,2</sup>

Nishan S. Kalupahana,<sup>3,4,6</sup> Kate J. Claycombe,<sup>7</sup> and Naima Moustaid-Moussa<sup>3,4,5,\*</sup>

<sup>3</sup>The University of Tennessee (UT) Obesity Research Center, <sup>4</sup>Department of Animal Science, and <sup>5</sup>UT Extension Family and Consumer Sciences Department, Family and Consumer Sciences, Knoxville, TN 37996; <sup>6</sup>Department of Physiology, Faculty of Medicine, University of Peradeniya, Peradeniya 20400, Sri Lanka; and <sup>7</sup>USDA Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, ND 58201

## ABSTRACT

Obesity is associated with the metabolic syndrome, a significant risk factor for developing type 2 diabetes and cardiovascular diseases. Chronic low-grade inflammation occurring in the adipose tissue of obese individuals is causally linked to the pathogenesis of insulin resistance and the metabolic syndrome. Although the exact trigger of this inflammatory process is unknown, adipose tissue hypoxia, endoplasmic reticular stress, and saturated fatty acid-mediated activation of innate immune processes have been identified as important processes in these disorders. Furthermore, macrophages and T lymphocytes have important roles in orchestrating this immune process. Although energy restriction leading to weight loss is the primary dietary intervention to reverse these obesity-associated metabolic disorders, other interventions targeted at alleviating adipose tissue inflammation have not been explored in detail. In this regard, (n-3) PUFA of marine origin both prevent and reverse high-fat-diet-induced adipose tissue inflammation and insulin resistance in rodents. We provide an update on the pathogenesis of adipose tissue inflammation and insulin resistance in obesity and discuss potential mechanisms by which (n-3) PUFA prevent and reverse these changes and the implications in human health. *Adv. Nutr.* 2: 304–316, 2011.

## Introduction

Obesity is a major health problem in the United States and worldwide. It is associated with metabolic syndrome, which is characterized by hyperglycemia, abdominal obesity, hypertension, elevated plasma TG, and reduced plasma HDL cholesterol levels (1). Individuals with metabolic syndrome frequently exhibit proinflammatory and prothrombotic metabolic profiles and are at a higher risk for developing type 2 diabetes and cardiovascular disease. Recent evidence has causally linked obesity and increased adiposity to the pathogenesis of metabolic syndrome and type 2 diabetes. Furthermore, adipose tissue dysfunction and inflammation have been identified as major players in these disorders.

Although energy restriction leading to weight loss is a successful dietary intervention for improving obesity-associated metabolic disorders, other dietary interventions such as ones

targeted at reducing adipose tissue inflammation, regardless of weight loss, have not been explored in detail. Long-chain (n-3) PUFA, namely EPA and DHA, have antiinflammatory properties (2). Moreover, they are well documented for reducing plasma TG (3,4) and these fatty acids exhibit antiobesity effects on humans (5) and rodents (6).

Currently, the effect of EPA and DHA on insulin sensitivity is not well characterized. Whereas these fatty acids consistently prevent the development of insulin resistance associated with high-fat (7,8) or high-sucrose (9) feeding in rodents, they do not significantly improve insulin sensitivity in individuals with type 2 diabetes (10–12). Nevertheless, some promising evidence suggests that EPA and DHA might help delay the progression of metabolic syndrome to type 2 diabetes (13). In this context, elucidating the mechanisms responsible for improvement of insulin sensitivity due to EPA and DHA might enhance the understanding of the pathophysiology of obesity-associated insulin resistance and identification of nutritionally relevant targets for the treatment of metabolic syndrome.

This review provides an update on the mechanistic aspects of the pathogenesis of adipose tissue inflammation

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\* To whom correspondence should be addressed. E-mail: moustaid@utk.edu.

and insulin resistance in obesity, followed by a summary of potential mechanisms by which EPA and DHA prevent and reverse these processes.

### Current status of knowledge

#### Adipose tissue dysfunction in obesity

White adipose tissue is the major site for storage of excess energy in the body. It is composed of adipocytes, an extracellular matrix (ECM),<sup>8</sup> vascular and neural tissues, and other cell types (14). These other cell types include preadipocytes, fibroblasts, stem cells, and immune cells such as macrophages and T lymphocytes. Adipose tissue secretes numerous bioactive peptides collectively known as adipokines (15,16). Examples include hormones involved in energy and glucose homeostasis such as leptin, adiponectin, resistin, apelin, and visfatin; chemokines such as monocyte chemoattractant protein (MCP)-1 and IL-8; other proinflammatory cytokines such as IL-6, IL-1, angiotensin-II, and TNF- $\alpha$ ; and antiinflammatory cytokines such as IL-10 (Table 1). Thus, adipose tissue is a dynamic endocrine organ with major roles in energy balance, glucose homeostasis, blood pressure regulation, and immune function (17).

Excessive TG accumulation within adipocytes, presumably linked to adipose tissue overload as a result of positive energy balance, leads to adipocyte hypertrophy and a dysregulation of adipokine secretory patterns. This has been primarily attributed to an imbalance between secretions of pro- compared to antiinflammatory adipokines. Thus, obesity is associated with a chronic low-grade inflammation in the adipose tissue (18,19). Although adipocytes are a source of proinflammatory cytokines in obesity (15,20), cells of the stromal vascular fraction such as preadipocytes (21), macrophages, and adipose stem cells can produce even higher levels of these cytokines (22). Major cell types that play key roles in the inflammatory response during onset of obesity are illustrated in Figure 1.

Although the exact trigger for the onset of adipose tissue inflammation is hitherto unknown, several possible mechanisms have been suggested and are discussed below. In a state of positive energy balance, adipose tissue expands to accommodate the storage of excess TG. Adipose tissue remodeling via degradation of the ECM and adipogenesis are 2 key processes in this expansion. Matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases play important roles in ECM degradation and adipose tissue remodeling (23,24). Defective adipose tissue expansion as a result of dysregulation of any of the above factors could lead to adipocyte injury, death, and inflammation (25). For

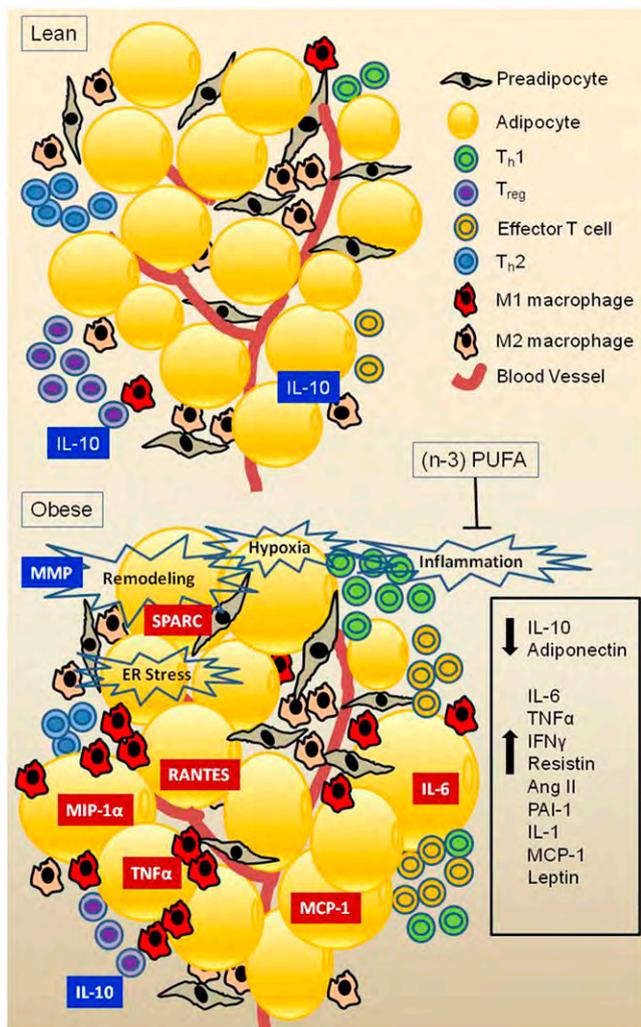
example, factors that promote adipose tissue fibrosis, such as secreted protein acidic and rich in cysteine, are associated with obesity and adipose tissue inflammation (25).

Rodent studies show that increasing adipose tissue mass without a similar magnitude increase in supporting vasculature could lead to tissue hypoxia, triggering the expression of hypoxia-inducible factor-1 and inflammatory genes (26). Similarly, oxygen partial pressure in subcutaneous adipose tissue negatively correlates with adiposity in humans (27). Thus, hypoxia could be a trigger for adipose tissue inflammation. Both animal and human studies support the role of adipose tissue endoplasmic reticulum (ER) stress as another key elicitor for subsequent inflammation in obesity (28–31).

**Table 1.** Major adipocytokines and their functions

Adipokine	Physiological effects	Reference
Leptin	Reduces energy intake and increases expenditure, angiogenesis, and hematopoiesis, immune functions	(130)
Adiponectin	Improves insulin sensitivity, antiinflammatory, antiatherogenic, promotes fatty acid oxidation	(131)
Resistin	Promotes insulin resistance	(132)
Angiotensin-II/angiotensinogen	Vasoconstriction, sodium and water retention, increases blood pressure, proinflammatory, promotes insulin resistance and induces lipogenesis	(133–135)
MCP-1	Promotes macrophage infiltration and insulin resistance, proinflammatory, chemotactic	(136,137)
TNF $\alpha$	Proinflammatory, promotes insulin resistance	(72)
PAI-1	Prothrombotic, proinflammatory	(138)
IL-6	Proinflammatory	(139)
IL-10	Antiinflammatory	(140)
Visfatin	Insulin-mimetic actions, cell proliferation	(141)
Apelin	Promotes glucose uptake, angiogenesis	(142)
Retinol-binding protein-4	Promotes insulin resistance	(143)
Vascular endothelial growth factor	Angiogenesis	(144)
Nerve growth factor	Neuronal development	(145)
IL-1	Proinflammatory	(146)
IL-1 receptor antagonist	Antiinflammatory	(146)
Vaspin	Insulin-sensitizing effects	(147)
Omentin	Regulates insulin action	(148)
Neuropeptide Y	Energy homeostasis, proliferation of preadipocytes	(149)
Hepcidin	Proinflammatory	(150)
IL-8	Proinflammatory, chemotactic	(151)
IL-18	Proinflammatory	(152)
Thrombospondin-1	Proinflammatory	(153)
Serum amyloid A	Proinflammatory, lipolytic	(154)
Chemerin	Impairs glucose tolerance	(155)

<sup>8</sup> Abbreviations used: AA, arachidonic acid; ALA,  $\alpha$ -linolenic acid; AMPK, AMP-activated protein kinase; ATM, adipose tissue macrophage; Ccr, C-C motif chemokine receptor; CD, cluster of differentiation; ECM, extracellular matrix; ER, endoplasmic reticulum; GPR, G protein-coupled receptor; IRS, insulin receptor substrate; JNK, jun N-terminal kinase; LA, linoleic acid; M1, classically activated macrophage; M2, alternatively activated macrophage; MCP, monocyte chemoattractant protein; MGL, macrophage galactose N-acetyl-galactosamine specific lectin; PAI, plasminogen activator inhibitor; PI3K, phosphatidylinositol 3-kinase; PSGL, P-selectin glycoprotein ligand; SOCS, suppressor of cytokine signaling; TAK, TGF- $\beta$  activated kinase; Th, helper T cell; TLR, Toll-like receptor.



**Figure 1** Obesity-associated immune cell infiltration of adipose tissue. Lean individuals exhibit higher ratios of M2:M1 macrophage, Th1:Th2 T cell, and regulatory:effector T cell. Excessive TG accumulation leads to adipose tissue remodeling, relative hypoxia, and ER stress, which trigger production of chemokines and changes in the above cell ratios, culminating in increased production of proinflammatory adipokines and reduced production of antiinflammatory adipokines.

Although there is evidence that adipose tissue expansion per se is an important initiator of the inflammatory processes during the development of obesity, other lines of evidence suggest that type of dietary fat is also an important factor in triggering this process. For example, the NF- $\kappa$ B pathway in the visceral adipose tissue is activated 2 h after consumption of a meal rich in SFA in rodents (32). Other studies have shown similar chronic effects of fatty acids in triggering inflammation in the adipose tissue (33–35). Given that these fatty acids are ligands for Toll-like receptors (TLR) 2 and 4, and because TLR2 and 4 are expressed in human adipocytes (36), it is likely that the effect of SFA on adipose tissue inflammation is mediated via these receptors. Indeed, obesity-induced adipose tissue inflammation is attenuated in mice with a mutation in TLR4 (37). Furthermore, TLR2

knockout mice are also protected from high-fat-diet-induced insulin resistance and  $\beta$ -cell dysfunction (38). Other receptors of the innate immune system such as Nod-like receptors are also implicated in obesity and high-saturated fat-associated adipose inflammation (39).

Conversely, there are studies showing that adipose tissue inflammation can be reduced without changing adipose mass (40,41). This is also consistent with our recent findings that EPA reverses high-fat-induced metabolic disorders and adipose inflammation (42). In this study, EPA supplementation reversed high-saturated fat diet-induced insulin resistance and hepatic steatosis and increased adipose tissue MCP-1 and plasminogen activator inhibitor (PAI)-1 levels in C57BL/6J mice. Moreover, high-fat feeding-induced adipose tissue inflammation is not completely resolved following energy restriction (43). Taken together, the current research suggests a role of dietary fat in the onset of adipose tissue inflammation.

Adipose tissue inflammation in obesity is characterized by macrophage infiltration (18,44) (Fig. 1). Adipose tissue macrophages (ATM) are classified into 2 main types. M1 or classically activated macrophages are stimulated by IFN $\gamma$  and LPS and produce proinflammatory cytokines such as TNF $\alpha$ , IL-6, and IL-1 and reactive oxygen species such as NO (Fig. 1). M2 or alternatively activated macrophages are activated by IL-4 and IL-13 and express antiinflammatory factors IL-10, TGF $\beta$ , IL-1 receptor antagonist-a, IL-4, and arginase (45,46). Phenotypically, murine ATM express the F4/80 antigen. The murine M1 ATM highly express cluster of differentiation (CD) 11c, and the M2 express macrophage galactose N-acetyl-galactosamine specific lectin (MGL) 1 (47). In contrast, human ATM express CD14, whereas CD11c is only poorly expressed. Human ATM also express CD206, CD209, and CD163 (47).

Obesity induces an M2 to M1 shift in ATM populations, characterized by a reduction in antiinflammatory IL-10 and arginase production and an increase in proinflammatory TNF $\alpha$  production (48) (Fig. 1). This increase in M1 ATM could be due to either a phenotypic switch from M2 to M1 or to additional recruitment of M1 macrophages from blood vessels. Lipotoxicity of macrophages seems to play a major role in the phenotypic switch of M2 to M1 (49). Detailed mechanisms of the M2 to M1 switch have previously been reviewed and summarized by Olefsky et al. (16). Briefly, TLR4 ligands such as SFA activate NF- $\kappa$ B and activator protein 1 transcription factors, leading to increased production of proinflammatory cytokines such as TNF $\alpha$ , IL-6, and IL-1, giving rise to the M1 phenotype. In the lean adipose tissue, this is prevented by repression of TLR4-responsive genes by nuclear receptor corepressor complexes. PPAR $\gamma$  along with IL-4 and IL-13 prevent the signal-dependent turnover of nuclear receptor corepressor and thus help maintain the M2 phenotype. However, it is worth noting that the M1:M2 polarization may be a more complex concept, because recent evidence has shown that in humans, even M2 macrophages are capable of producing excessive amounts of proinflammatory cytokines in obesity (50).

Evidence for M1 recruitment originated from studies showing increased MGL1<sup>-</sup> C-C motif chemokine receptor (Ccr)<sup>+</sup> macrophages recruited around necrotic adipocytes in high-fat-diet-fed mice, whereas the MGL<sup>+</sup> ATM levels remained unchanged (51). Adipose tissue from obese animals expresses high levels of chemokines such as MCP-1, macrophage inflammatory protein-1 $\alpha$ , and RANTES (Regulated Upon Activation, Normal T-Cell Expressed and Secreted); chemokine receptors such as Ccr2 and Ccr5 (18); and adhesion molecules such as P-selectin glycoprotein ligand (PSGL)-1; (52). The expression of these chemokines, chemokine receptors, and adhesion molecules play a major role in the recruitment of macrophages to adipose tissue in obesity. Indeed, mice overexpressing MCP-1 in the adipose tissue have higher macrophage infiltration, whereas MCP-1 knockout mice are protected from high-fat-diet-induced ATM infiltration (53). In agreement with these findings, Ccr2-deficient mice exhibit low ATM numbers (54), whereas PSGL-1 knockout mice are protected from high-fat-diet-induced adipose inflammation (52). In contrast, however, macrophage inflammatory protein-1 $\alpha$ -deficient mice are not protected against high-fat-diet-induced macrophage infiltration into adipose tissue (55). Thus, although MCP-1 appears to be a key mediator of the initiation of adipose tissue inflammation in obesity, the exact mechanisms remain to be elucidated. It is likely that adipocyte hypertrophy, a hallmark of inflamed adipose tissue, is critical in the pathogenesis of these metabolic disorders. This is further supported by clinically relevant findings that adipocyte size positively correlates with MCP-1 expression in humans (56,57).

Recent evidence also points toward involvement of T cells in obesity-associated adipose tissue inflammation (58). Nishimura et al. (59) showed that CD8 (+) effector T cells infiltrate the adipose tissue in high-fat-fed mice, with a concurrent reduction in CD4 (+) helper (Th) and regulatory T cells. Moreover, these changes occur before the adipose tissue infiltration with macrophages. The adipose tissue infiltration of macrophages is prevented by genetic depletion of CD8 (+) T cells. Feuerer et al. (60) showed that the number of Treg cells in the white adipose tissue of obese mice is significantly lower than in lean ones. Winer et al. (61) showed that obese mice have a higher Th1:Th2 ratio promoting IFN $\gamma$  secretion from adipose tissue (Fig. 1). Taken together, this suggests that T cells are early modulators of adipose tissue inflammation in obesity. The cytokine profile of these T cells could play an important role in determining the M1/M2 phenotype of ATM.

### Molecular mechanisms of insulin resistance

Insulin resistance is defined as an inadequate response by insulin-sensitive tissues (liver, skeletal muscle, and adipose tissue) to normal circulating levels of insulin (62). At physiological levels, insulin inhibits hepatic glucose production, promotes skeletal muscle glucose uptake, and inhibits lipolysis. Insulin resistance leads to impairments in insulin-mediated suppression of hepatic glucose production, skeletal muscle glucose disposal, and inhibition of lipolysis, leading

to relative hyperglycemia and increased plasma levels of FFA (16). In response to the relative hyperglycemia, there is a compensatory response by the pancreatic  $\beta$ -cells, which secrete more insulin. This hypersecretion of insulin in turn increases skeletal muscle glucose uptake and inhibits hepatic glucose production to maintain normoglycemia. Thus, insulin-resistant individuals maintain normoglycemia through overproduction and secretion of higher insulin levels (63). Long-term insulin resistance and hypersecretion of insulin eventually leads to pancreatic  $\beta$ -cell failure. These events result initially in prediabetes and glucose intolerance and later progress to hyperglycemia and type 2 diabetes (63).

Insulin exerts its physiological actions on insulin-sensitive tissues via activation of a cascade of intracellular signaling events, all of which have been previously reviewed (64,65). Briefly, insulin binds to the insulin receptor, triggering its autophosphorylation as well as tyrosine phosphorylation of downstream substrates, including the insulin receptor substrate (IRS). The latter is a critical step in eliciting IRS binding to Src-homology-2 domain of the regulatory subunit of phosphatidylinositol 3-kinase (PI3K). This binding subsequently activates the catalytic subunit of PI3K, which in turn catalyzes the formation of lipid second messenger PIP<sub>3</sub>. Binding of this lipid moiety to proteins with pleckstrin-homology domains leads to their activation. Additionally, activation of 3-phosphoinositide-dependent protein kinase 1, leads to activation of Akt/protein kinase B. Akt/protein kinase B is a serine/threonine kinase that targets several downstream proteins. Moreover, phosphorylation of Rab small GTPases and inactivation of AS160 by Akt initiates cytoskeletal reorganization and results in translocation of glucose transporter-4 into the cell membrane and facilitates glucose entry into cells.

Akt also phosphorylates and deactivates glycogen synthase kinase 3, which leads to activation of glycogen synthase and subsequent glycogen synthesis. Akt also regulates transcription of several genes involved in gluconeogenesis and lipogenesis via control of winged helix or forkhead box O class of transcription factors. A well-studied example is inhibition by Akt of the forkhead box O-mediated activation of hepatic gluconeogenic genes in the liver (64). Thus, the net effect of these signaling and activation cascades is increased glucose entry into cells as well as increased flux of glucose into intra-cellular metabolic pathways of skeletal muscle and adipose tissue and reduced hepatic gluconeogenesis.

Downregulation of insulin receptor protein level, as seen in obesity, can result in insulin resistance (64). Defective insulin signaling at various levels of the above cascade is also known to be associated with insulin resistance. A reduction in IRS protein levels is also associated with insulin resistance. Hyperinsulinemia itself can reduce IRS protein via transcriptional regulation (65). Suppressor of cytokine signaling (SOCS)-3 blocks the interaction between insulin receptor and IRS and contributes to insulin resistance (66). Serine phosphorylation of IRS by FFA, cytokines (67), and activation of NF- $\kappa$ B-mediated inflammatory pathways (68) is also known to induce insulin resistance. SOCS1 and 3 are

also known to induce degradation of IRS (69). Further downstream, higher expression of the regulatory subunit of PI3K is also associated with insulin resistance (70).

### Proposed mechanisms for obesity-induced insulin resistance

Obesity induces insulin resistance in skeletal muscle, liver, and adipose tissue (16). Several models have been put forward to explain mechanisms of obesity-induced insulin resistance. The chronic low-grade inflammation occurring in adipose tissue is considered to be a major factor in the pathogenesis of obesity-induced insulin resistance. There are several lines of evidence to support this model. First, adipose-specific overexpression of proinflammatory cytokines such as MCP-1 or Agt induces whole-body insulin resistance (53,71). Second, neutralization or knockdown of inflammatory mediators such as TNF $\alpha$ , MCP-1, Ccr-2, and PSGL-1 protects rodents from high-fat-diet-induced insulin resistance (52–54,72). Finally, overexpression of antiinflammatory adipokines such as adiponectin protects rodents from high-fat-diet-induced insulin resistance (73).

Increased proinflammatory cytokines can induce insulin resistance by several mechanisms. As outlined earlier, proinflammatory cytokines can induce SOCS3 expression, which in turn can inhibit insulin signaling by inhibiting IRS action (66). Proinflammatory cytokines also activate numerous intracellular serine kinases such as jun N-terminal kinase (JNK) and inhibitor of  $\kappa$ B kinase. These serine kinases can also inhibit insulin signaling at various levels (68). Indeed, JNK1 or inhibitor of  $\kappa$ B kinase- $\beta$  knockout mice and mice with adipose-specific JNK inactivation are protected from insulin resistance (74–76). Finally, increased circulating FFA levels due to adipose tissue insulin resistance can in turn inhibit insulin signaling via serine phosphorylation of IRS (67) and lead to insulin resistance in skeletal muscle and liver.

Although the imbalance of pro- and antiinflammatory adipokines can induce insulin resistance via paracrine effects, the endocrine effects of these adipokines are especially important in the development of insulin resistance in skeletal muscle and liver (72). For example, circulating levels of adiponectin, an adipokine exclusively secreted by the adipose tissue, is positively correlated with insulin sensitivity in both humans and rodents (77). Moreover, individuals with high-plasma adiponectin levels have a lower risk of developing type 2 diabetes (78). Finally, abdominal adiposity correlates with plasma C-reactive protein levels, indicating that systemic markers of inflammation are also increased with obesity (79). Whereas adipose tissue inflammation in obesity plays a key role in the development of insulin resistance, adipose inflammation in the absence of obesity does not seem to induce insulin resistance (80). Thus, it is important to use mouse models with at least some degree of obesity when studying the contribution of individual inflammatory mediators to insulin resistance.

Increased lipid deposition in skeletal muscle and liver is also considered to be a factor linked to the pathogenesis of

insulin resistance (62). Indeed, obese, insulin-sensitive individuals have lower skeletal muscle and liver lipids than obese, insulin-resistant individuals (81). This ectopic lipid deposition is attributed to the inability of the adipose tissue (mainly subcutaneous) to store excess energy due to reduced differentiation/remodeling capacity. This is also characterized by increased visceral fat mass. Although the exact mechanism of these defects in adipogenesis/remodeling is not known, proinflammatory cytokines such as TNF $\alpha$  are implicated because of their known inhibitory effects on adipogenesis (82), in a PPAR $\gamma$ -dependent manner. Conversely, PPAR $\gamma$  agonists such as thiazolidinediones are known to increase both adipogenesis and insulin sensitivity. Increased lipid accumulation in the liver and skeletal muscle is associated with increased fatty acid flux, which leads to excessive accumulation of fatty acid intermediates such as ceramide (62). These lipid intermediates activate intracellular serine kinases that can lead to inhibition of insulin signaling. Ceramide can also directly inhibit Akt (83). Indeed, pharmacological inhibition of ceramide synthesis protects rodents from obesity-associated insulin resistance (84).

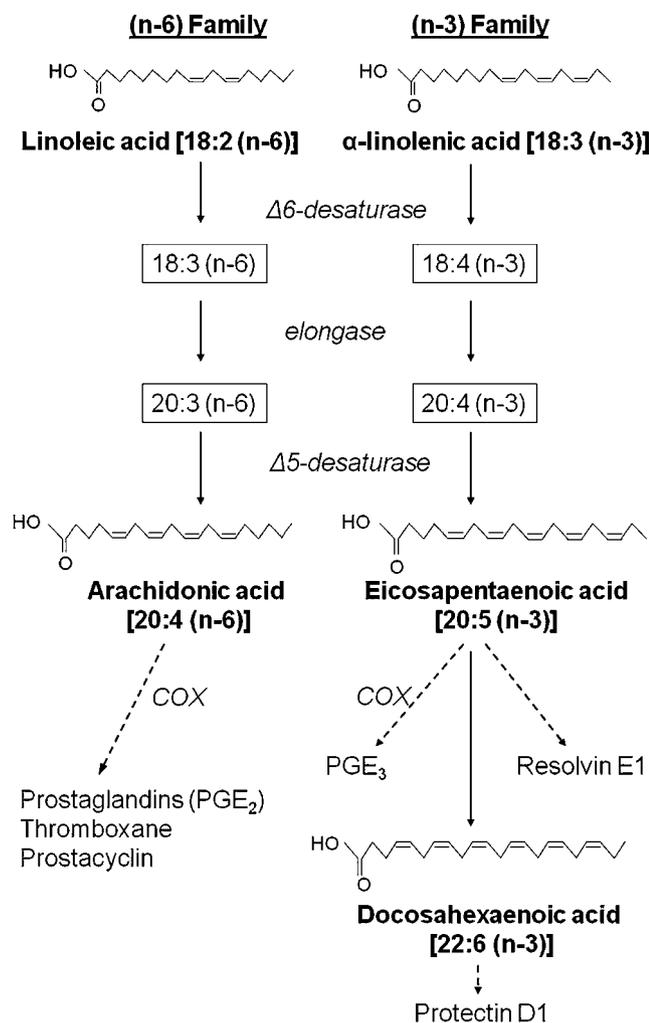
### Long-chain (n-3) PUFA for improvement of insulin resistance and metabolic derangements in obesity

(n-3) and (n-6) PUFA are the 2 main classes of essential fatty acids. Linoleic acid (LA) is the parent long-chain (n-6) PUFA, which can be converted into arachidonic acid (AA) (Fig. 2).  $\alpha$ -Linolenic acid (ALA) is the parent (n-3) fatty acid, which can be converted to EPA and DHA. The latter fatty acids are found primarily in foods of marine origin such as oily fish. The ratio of (n-6):(n-3) PUFA in the Western diet ranges from about 10:1 to 20:1 (85), whereas in countries with a relatively higher fish consumption such as Japan, this ratio is 4:1 (86). Dietary intake of these fatty acids affects the proportion of AA:EPA ratio in phospholipids, which affects cardiovascular disease risk (87). The (n-3) index is a measure of erythrocyte EPA+DHA:total fatty acid ratio, which has been proposed to be used as a cardiovascular disease risk factor. Individuals with a low (n-3) index have a higher risk of cardiac events (88).

The TG-lowering and cardioprotective actions of EPA and DHA are well established. Additionally, rodent studies show that EPA and DHA also prevent and reverse insulin resistance associated with high-fat (42) or high-sucrose feeding (9). Because insulin resistance associated with these dietary conditions is due to defects in adipose tissue, skeletal muscle, and hepatic function (discussed above), it is particularly important to understand how EPA and DHA modulate the functions of these organs.

### Effects of EPA and DHA on adipose tissue function

EPA and DHA reduce adiposity in humans (89) especially when combined with energy restriction (5). These fatty acids also prevent the development of high-fat-diet-induced adiposity and adipocyte hypertrophy in rodents (42,90). There are 2 possible mechanisms for these antiobesity effects of



**Figure 2** Metabolism of (n-3) and (n-6) fatty acids. LA and ALA are the parent (n-6) and (n-3) long-chain PUFA. LA is converted to AA and ALA is converted to EPA and DHA. AA-derived eicosanoids are proinflammatory, whereas EPA-derived ones are less so. EPA and DHA metabolites such as resolvins and protectins have important roles in the resolution of inflammation. COX, cyclooxygenase.

EPA and DHA. First, these fatty acids are known to increase fatty acid oxidation in liver, adipose tissue (91), and small intestine (92) in rodents *in vivo* and adipocytes (93) and myotubules (94) *in vitro*. Fish oil also increases fatty acid oxidation in humans with a reduction in respiratory quotient (89). Second, they are known to inhibit hepatic lipogenesis (Fig. 3). Both these processes shift the balance of fatty acid metabolism toward oxidation rather than storage. EPA and DHA activate AMP-activated protein kinase (AMPK) in adipose tissue and cultured adipocytes, which could be a mechanism for their effect on fatty acid oxidation (95,96). Further, these PUFA are also known to induce mitochondrial biogenesis (3) (Fig. 4).

Although it is possible that improvements of systemic insulin resistance due to EPA and DHA are secondary to reduction in adipose mass, this could also be due to direct actions of these fatty acids in improving adipose tissue function.

Indeed, some studies have shown EPA- and DHA-mediated insulin sensitivity is preserved even in the presence of increased adipose mass (97). EPA and DHA modulate adipokine secretion from adipose tissue (Figs. 3 and 4). They increase plasma adiponectin levels in obese humans (98,99) and rodents (100), which could be a potential mechanism by which EPA and DHA improve insulin sensitivity. This effect of EPA and DHA on adiponectin is PPAR $\gamma$ -dependent, because adiponectin is not elevated in response to fish oil in mice lacking PPAR $\gamma$  (101). They also induce leptin and visfatin secretion and reduce the expression of several proinflammatory cytokines from the adipose tissue, including TNF $\alpha$ , IL-6, MCP-1, and PAI-1 (42,102–104). Current evidence suggests that these antiinflammatory actions of EPA and DHA play a major role in their insulin-sensitizing effects.

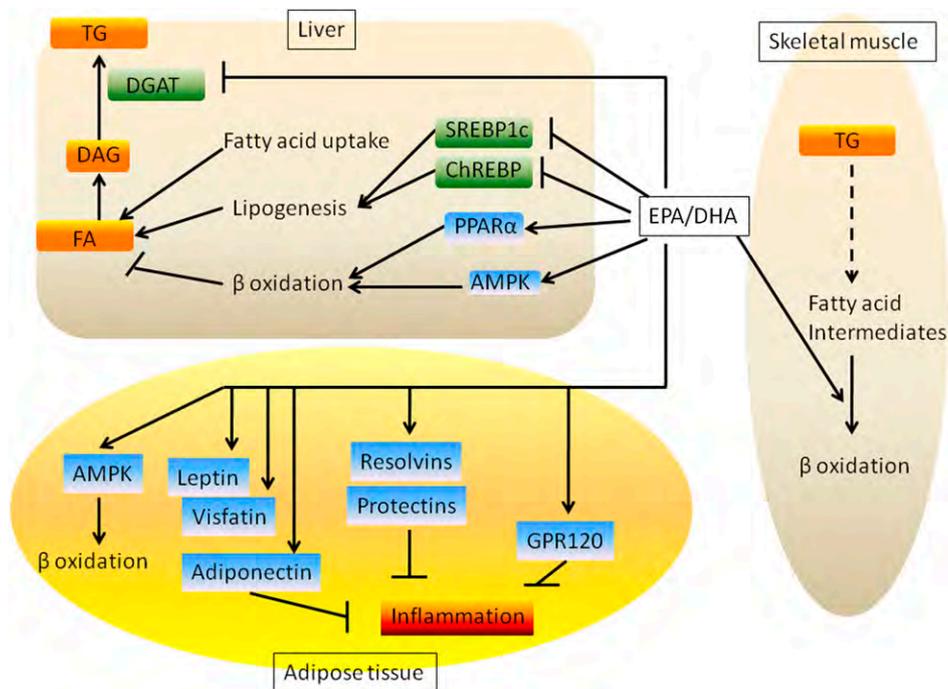
ATM infiltration and phenotypic switch are causally linked to insulin resistance in obesity (discussed previously in this review). EPA and DHA prevent high-fat-diet-induced ATM infiltration in mice (105). Production of proinflammatory cytokines by macrophages is dependent on activation of the NF- $\kappa$ B and JNK pathways. EPA and DHA bind to G protein-coupled receptor (GPR) 120, and inhibit NF- $\kappa$ B and JNK, attenuating this response (106) (Fig. 4). The importance of this receptor, which is present in both adipocytes and macrophages, is highlighted by the finding that the EPA-mediated improvement in insulin sensitivity is absent in mice lacking GPR120. More specifically, TGF- $\beta$  activated kinase (TAK)-1 is necessary for the activation of NF- $\kappa$ B and JNK, which in turn is dependent upon association of TAK-1 with TAK-1 binding protein-1. EPA or DHA binding to GPR120 leads to its internalization along with  $\beta$ -arrestin2. This complex associates with TAK-1 binding protein-1 and prevents the activation of TAK-1 binding protein-1, thereby preventing activation of NF- $\kappa$ B and JNK. Another GPR, GPR40, is also activated by long-chain PUFA (107).

Recent evidence has highlighted the role of EPA and DHA in resolving inflammation, through mechanisms involving EPA-derived resolvin E1 and DHA-derived protectin D1 (108). In high-fat-fed mice, protectin D1 is lacking in the adipose tissue and skeletal muscle. Moreover, transgenic restoration of (n-3) PUFA and protectin D1 prevents the high-fat-diet-induced insulin resistance, highlighting the important role of this DHA derivative (109).

AA-derived eicosanoids such as PGE $_2$  and thromboxane A $_2$  are proinflammatory, whereas EPA-derived ones such as PGE $_3$  are less inflammatory. Because higher EPA and DHA intakes result in higher incorporation into membrane phospholipids at the expense of AA, it is possible that EPA and DHA supplementation reduces production of AA-derived eicosanoids. This can also contribute to antiinflammatory effects of these fatty acids. Indeed, we previously showed that both murine and human adipocytes secrete prostaglandins (110) and EPA significantly reduced adipose PGE $_2$  secretion and antagonized AA-induced PGE $_2$  secretion from cultured adipocytes (111) (Fig. 4).

Adipose stem cells are a major source of proinflammatory cytokines in adipose tissue (22). Recently, we tested

**Figure 3** Integrated effects of EPA and DHA on liver, skeletal muscle, and adipose tissue metabolism. EPA and DHA promote hepatic fatty acid oxidation and suppress lipogenesis. This leads to reduced accumulation of TG in the liver. These fatty acids also increase adipose tissue fatty acid oxidation and increase secretion of adiponectin, leptin, and visfatin. EPA and DHA also alleviate adipose tissue inflammation via GPR120 and resolvins/protectins. In the skeletal muscle, EPA and DHA promote fatty acid oxidation, thereby preventing accumulation of fatty acid intermediates. All these mechanisms account for the EPA- and DHA-mediated improvement in insulin sensitivity.



whether EPA and/or stearidonic acid [18:4 (n-3), EPA precursor] inhibit LPS-mediated inflammation in mouse adipose stem cells (112). Our results demonstrated that both stearidonic acid and EPA significantly reduced LPS-induced IL-6 secretion and IL-6 mRNA expression via TLR2 and NF- $\kappa$ B-mediated pathways (Fig. 4).

### EPA and DHA and hepatic insulin sensitivity

The effect of (n-3) PUFA, mainly EPA, on lowering plasma TG is well established. This effect is at least in part due to their ability to inhibit hepatic enzyme diacylglycerol acyltransferase (113), which catalyzes the final reaction of TG synthesis. In addition to this TG-lowering effect, EPA and DHA also prevent the development of hepatic steatosis associated with high-saturated fat feeding in rodents (114).

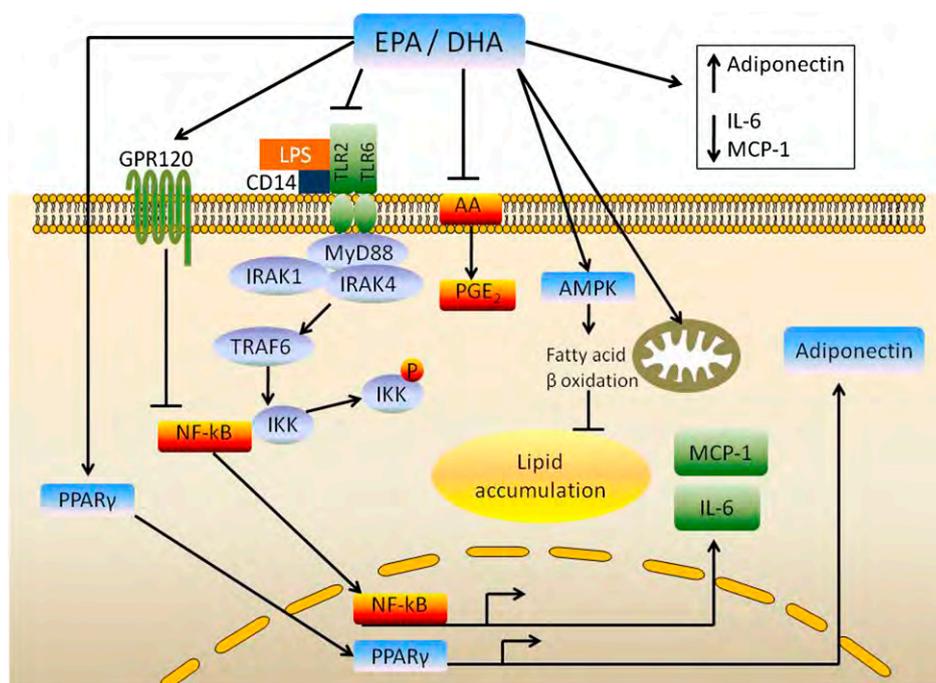
Lipid accumulation in the liver depends on nonesterified fatty acid delivery to the liver, de novo lipogenesis, and the rate of fatty acid oxidation (Fig. 3). In obesity, there is a net increase in fatty acid availability, promoting lipid deposition in the liver. Moreover, lipogenic gene transcription factors such as sterol regulatory element-binding protein-1c, are expressed at a higher level in obesity (115). This leads to increased expression of hepatic lipogenic genes such as fatty acid synthase and stearoyl-CoA desaturase 1 (116). Further, obesity is also associated with suppression of PPAR $\alpha$  (115) leading to reduced fatty acid oxidation (116). All these processes are linked to the development of hepatic steatosis. Excessive lipid accumulation in the liver leads to hepatic insulin resistance and blunting of insulin-mediated suppression of hepatic glucose production.

EPA reduces lipogenesis and increases fatty acid oxidation (117), preventing lipid accumulation in the liver, leading to improvements in hepatic insulin resistance (Fig. 3). Moreover,

EPA reduces lipogenesis via inhibition of lipogenic transcription factors such as sterol regulatory element-binding protein-1c, nuclear factor-Y (118), and carbohydrate-responsive element-binding protein (119). EPA stimulates fatty acid oxidation via activation of PPAR $\alpha$  (116,120–122) and AMPK (123). PPAR $\alpha$  is required for EPA's beneficial effects on hepatic insulin sensitivity, as evidenced by a lack of EPA effect in restoring hepatic insulin sensitivity in PPAR $\alpha$  null mice fed a high-fat diet (124). Interestingly, these mice continue to exhibit low-plasma TG levels, concomitant with diacylglycerol accumulation in the liver, suggesting that EPA exerts a PPAR $\alpha$ -independent effect on hepatic diacylglycerol acyltransferase. AMPK $\alpha$ 2 is another signaling enzyme, coordinately regulated with PPAR $\alpha$  during fat oxidation. As expected, and in line with the PPAR $\alpha$  null mice phenotype, AMPK $\alpha$ 2 null mice do not exhibit EPA's beneficial effects on improvement in hepatic insulin sensitivity (125).

### EPA and DHA and skeletal muscle metabolism

TG accumulation in skeletal muscle fibers has been linked to insulin resistance (discussed previously). Proposed mediators include increased fatty acid availability and impaired fatty acid oxidation in the skeletal muscle. The latter is also associated with accumulation of fatty acid intermediates such as diacylglycerol and ceramides. Exposure of myotubules to EPA enhances glucose uptake (126), indicating increased insulin sensitivity. EPA also protects from the development of high-fat-induced skeletal muscle insulin resistance in vivo (127), with improvements in muscle glycogen synthesis (100). Interestingly, some studies have shown that EPA increases TG accumulation, along with increases in fatty acid  $\beta$  oxidation (Fig. 3) and improvements in skeletal muscle insulin sensitivity both in vitro (94) and in vivo (128).



**Figure 4** Mechanisms by which EPA and DHA modulate adipose tissue function. LPS binds to TLR2/TLR6 complex via CD14. The binding of LPS to TLR2 stimulates IL-6 gene transcription by activation and recruitment of myeloid differentiation factor 88 (MyD88) and IL-1 receptor-associated kinase (IRAK)-4. The latter phosphorylates IRAK-1, followed by activation of TNF receptor-associated factor (TRAF)-6 and NF- $\kappa$ B. The latter translocates into the nucleus and activates IL-6 gene transcription. EPA and DHA inhibit LPS-induced IL-6 gene expression in adipose stem cells via regulation of IRAK1 and NF- $\kappa$ B pathways. EPA and DHA also inhibit the NF- $\kappa$ B pathway via GPR120. These fatty acids also activate PPAR $\gamma$ , leading to

increased production of adiponectin, in addition to their known antiinflammatory effects mediated by antagonism of the (n-6) fatty acid arachidonic acid (AA). Finally, EPA and DHA activate AMPK and mitochondrial biogenesis, both of which increase fatty acid oxidation and lower lipid accumulation in adipocytes.

Because EPA and DHA also reduce skeletal muscle ceramide content (100), it is possible that their effect on maintaining skeletal muscle insulin sensitivity is related to their ability to normalize fatty acid oxidation with lower accumulation of fatty acid intermediates. SFA induce skeletal muscle insulin resistance via activation of the NF- $\kappa$ B pathway (129). Because EPA and DHA inhibit this pathway in other tissues, it will be interesting to determine whether EPA and DHA inhibit this pathway in the skeletal muscle and subsequently prevent the SFA-mediated insulin resistance.

Although EPA and DHA consistently improve insulin resistance in rodent models of obesity, this is not the case for humans with type 2 diabetes. In a previous review of clinical studies on the use of (n-3) PUFA for glycemic control of individuals with type 2 diabetes, possible causes for this lack of effect were suggested to be due to inadequate dose and lack of control of the background diet (12). It is also possible that EPA and DHA delay the progression of metabolic syndrome and/or prediabetes to type 2 diabetes. Further clinical studies testing this hypothesis are warranted.

### Conclusions

In summary, obesity and increased adiposity are associated with a chronic, low-grade inflammation in the adipose tissue. Current evidence suggests that adipose tissue hypoxia, immune cell chemotaxis to adipose tissue followed by subsequent activation, ER stress, and SFA-mediated activation of innate immune receptors play a role in the trigger of this inflammatory process. ATM and T lymphocytes are the 2 key immune cell types that orchestrate these processes. Adipose tissue inflammation and associated hepatic steatosis

is causally linked to the development of skeletal muscle and systemic insulin resistance.

EPA and DHA prevent excessive adiposity and insulin resistance in rodents. Mechanistically, this is related to the ability of these fatty acids to increase hepatic, skeletal muscle, and adipose tissue fatty acid oxidation and their ability to reduce lipogenesis. EPA and DHA also have important antiinflammatory properties that modulate adipose tissue inflammation via GPR120-mediated suppression of macrophage proinflammatory cytokine secretion, resolvins, and protectin-mediated resolution of inflammation. Through modulation of adipokine secretion, these fatty acids also favor insulin sensitivity. Further studies in obese humans are warranted to study whether these fatty acids can prevent and reverse the progression of metabolic syndrome to type 2 diabetes.

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N.S.K. and N.M.-M. analyzed data and wrote the paper; K.J.C. reviewed and edited the paper; N.M.-M. had primary responsibility for final content. All authors read and approved the final manuscript.

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