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REVIEW

Cellular and molecular effects of *n*−3 polyunsaturated fatty acids on adipose tissue biology and metabolism

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ABSTRACT

Adipose tissue and its secreted products, adipokines, have a major role in the development of obesity-associated metabolic derangements including Type 2 diabetes. Conversely, obesity and its metabolic sequelae may be counteracted by modulating metabolism and secretory functions of adipose tissue. LC-PUFAs (long-chain polyunsaturated fatty acids) of the *n*−3 series, namely DHA (docosahexaenoic acid; $C_{22:6n-3}$) and EPA (eicosapentaenoic acid; $C_{20:5n-3}$), exert numerous beneficial effects, such as improvements in lipid metabolism and prevention of obesity and diabetes, which partially result from the metabolic action of *n*−3 LC-PUFAs in adipose tissue. Recent studies highlight the importance of mitochondria in adipose tissue for the maintenance of systemic insulin sensitivity. For instance, both *n*−3 LC-PUFAs and the antidiabetic drugs TZDs (thiazolidinediones) induce mitochondrial biogenesis and β-oxidation. The activation of this 'metabolic switch' in adipocytes leads to a decrease in adiposity. Both *n*−3 LC-PUFAs and TZDs ameliorate a low-grade inflammation of adipose tissue associated with obesity and induce changes in the pattern of secreted adipokines, resulting in improved systemic insulin sensitivity. In contrast with TZDs, which act as agonists of PPARγ (peroxisome-proliferator-activated receptor-γ) and promote differentiation of adipocytes and adipose tissue growth, *n*−3 LC-PUFAs affect fat cells by different mechanisms, including the transcription factors PPARα and PPARδ. Some of the effects of *n*−3 LC-PUFAs on adipose tissue depend on their active metabolites, especially eicosanoids. Thus treatments affecting adipose tissue by multiple mechanisms, such as combining *n*−3 LC-PUFAs with either caloric restriction or antidiabetic/anti-obesity drugs, should be explored.

INTRODUCTION

Obesity represents an increasing problem of health care. Obesity leads to various chronic morbidities, including Type 2 diabetes, dyslipidaemia and hypertension, i.e. major components of the MS (metabolic syndrome). The strongest correlation exists between accumulation of body fat and diabetes [1,2]. This suggests the importance of AT (adipose tissue) metabolism and AT-derived factors (fatty acids and adipokines) in the development

Key words: adiponectin, diabetes, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), insulin, obesity.

Abbreviations: ALA, α-linolenic acid; AMPK, AMP-activated protein kinase; AT, adipose tissue; BAT, brown AT; CB1 receptor, cannabinoid type 1 receptor; COX, cyclo-oxygenase; CPT-1, carnitine palmitoyltransferase-1; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GLUT-4, glucose transporter-4; HF, high-fat; IL, interleukin; LC, long-chain; MCP-1, monocyte chemoattractant protein-1; MS, metabolic syndrome; NEFA, non-esterified fatty acid; NRF-1, nuclear respiratory factor-1; POP, persistent organic pollutant; PPAR, peroxisome-proliferator-activated receptor; PGC-1α, PPARγ coactivator-1α; PLA_2, phospholipase A_2; PUFA, polyunsaturated fatty acid; SCD-1, stearoyl-CoA desaturase-1; SREBP-1, sterol-regulatory-element-binding protein-1; TNF-α, tumour necrosis factor-α; TZD, thiazolidinedione; UCP, uncoupling protein; aP2-*Ucp1* transgenic mice, mice harbouring ectopic expression of UCP-1 in WAT (white AT).

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Table 1 Nomenclature of $n-3$ LC-PUFAs
Adapted from Table 1.1 in [175].

Name		Abbreviation		
Trivial	Chemical	Carboxyl reference	Omega reference	Other
Linolenic acid	9,12,15-Octadecenoic acid	$C_{18:3(\Delta 9,12,15)}$	$C_{18:3n-3}$ or $C_{18:3(\omega 3)}$	ALA
Eicosapentaenoic acid, icosapentaenoic acid or timnodonic acid	5,8,11,14,17-Eicosapentaenoic acid	$C_{20:5(\Delta 5,8,11,14,17)}$	$C_{20:5n-3}$ or $C_{20:5(\omega 3)}$	EPA
Docosahexaenoic acid	4,8,12,15,19-Docosahexaenoic acid	$C_{22:6(\Delta 4,8,12,15,19)}$	$C_{22:6n-3}$ or $C_{22:6(\omega 3)}$	DHA

Table 2 Beneficial effects of $n-3$ LC-PUFAs in the prevention and reversal of components of the MS
*In adult humans. HDL, high-density lipoprotein; LDL, low-density lipoprotein; CVD, cardiovascular disease; ICHS, ischaemic coronary heart disease.

Aberration	Effect of $n-3$ LC-PUFA	Effective dose of EPA and DHA (g/day)*	Reference
Obesity	Well-documented anti-obesity effects in animal studies	—	[7,24,29,115,130,161]
	Moderate body-fat-lowering effects in small cohorts of human subjects	2–3	[22,23,162]
Dyslipidaemia	Reductions in plasma triacyglycerols by 20–30 % in most animal and human studies	1–4	[163,164]
	Improvements in plasma lipoprotein profile, namely increases in HDL-cholesterol and decreases in LDL-cholesterol in some studies	1.5–4	[165,166]
CVD	Lower incidence of ICHS in large epidemiological and prospective studies; anti-arrhythmic effects	0.2–1	[167,168]
	Secondary prevention of ICHS and CVD mortality	0.5–1.8	[167–169]
	Blood-pressure-lowering effect	3–6	[169]
	Slower progression of atherosclerosis	1.5–5	[169]
Insulin resistance	Prevention of diet-induced insulin resistance in animal studies	—	[9,18,19,138,170–174]
	Improvements of insulin sensitivity and glucose homoeostasis in healthy individuals	2	[21]
Type 2 diabetes	Without consistent effects	—	Reviewed in [164,166]

of systemic insulin resistance, the key event in the pathophysiology of the MS [1,2]. Insulin resistance most probably results from increased accumulation of lipids in the peripheral tissues (lipotoxicity) due to enhanced release of fatty acids from hypertrophic fat cells.

Increased physical activity and dietary manipulation in patients with impaired glucose tolerance has been shown to lower the incidence of Type 2 diabetes by 60 % [3,4]. The quality of dietary lipids is important, in particular the LC-PUFAs (long-chain polyunsaturated fatty acids) of the $n-3$ (omega-3) series DHA (docosahexaenoic acid; $C_{22:6n-3}$) and EPA (eicosapentaenoic acid; $C_{20:5n-3}$; for nomenclature of $n-3$ PUFAs, see Table 1), which are abundant in sea fish, lower triacylglycerols (triglycerides) while increasing HDL (high-density lipoprotein)-cholesterol levels in plasma, prevent the development of heart disease and exert anti-inflammatory properties in humans (reviewed in [5], and see Table 2). Studies in rats and mice fed an HF (high-fat) or lipogenic sucrose-rich diet report a counteraction of the development of both obesity and insulin resistance by $n-3$ LC-PUFAs. Several studies have demonstrated a decrease in adiposity in obese humans and improved glucose metabolism in healthy lean individuals after $n-3$ LC-PUFA supplementation. However, the findings on the reversal of already established insulin resistance and obesity by $n-3$ LC-PUFAs are ambiguous (see Table 2 for references). Conflicting results concerning the reversal of established insulin resistance were also obtained using laboratory mice [6,7] and rats [8–11]. The reason for poor effectiveness of $n-3$ LC-PUFA administration in the reversal of insulin resistance remains to be established. However, $n-3$ LC-PUFAs decreased plasma triacylglycerol levels without adverse side-effects even in patients with diabetes [12,13].

The high potency of $n-3$ LC-PUFAs to regulate metabolism may reflect the formation of their metabolites acting as signalling molecules. The effects of $n-3$ LC-PUFAs also depend on the ratio of dietary $n-6$ (omega-6) to $n-3$ PUFAs, which was lower in the diet of ancient hunter-gatherers compared with that of modern humans and is still increasing in affluent societies [14,15]. It is also important to discriminate between the $n-3$ LC-PUFAs DHA and EPA and their precursor ALA (α-linolenic acid; $C_{18:3n-3}$), as $n-3$ LC-PUFAs usually exert much stronger effects.

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The metabolic effects of $n-3$ LC-PUFAs primarily result from their interactions with several organ systems. The liver is involved in (i) the hypolipidaemic effect, due to a decrease in lipogenesis, lower formation of triacylglycerols, and their lower release as VLDLs (very-low-density lipoproteins) into circulation, and the increase in fatty acid oxidation *in situ* [9,16,17]; and (ii) the prevention of glucose intolerance in animals fed an HF diet, due to the suppression of gluconeogenesis by $n-3$ LC-PUFAs. EPA, but not DHA, is probably the main hypolipidaemic constituent of fish oils, due to its stimulatory effect on mitochondrial fatty acid oxidation in the liver [17]. The skeletal muscle, quantitatively the most important site of whole-body glucose utilization, is involved in the insulin-sensitizing effect of $n-3$ LC-PUFAs, due to its increased glucose uptake and utilization (reviewed in [9]). The prevention of triacylglycerol accumulation in non-ATs represents a common feature of $n-3$ LC-PUFA feeding in all animal models of obesity and insulin resistance [18,19]. These fatty acids probably induce a switch in substrate metabolism, namely an increase in whole-body fat oxidation and a decrease in carbohydrate oxidation [20–23]. $n-3$ LC-PUFAs affect the development of AT, as well as its metabolism and secretory functions. Recent studies document the major role of AT in the development of the MS and suggest the possibility of ameliorating obesity and its metabolic consequences by modulating the metabolism and secretory functions of AT. Therefore the aim of the present review is to summarize the effects of $n-3$ LC-PUFAs mainly on AT as a basis for understanding better the mechanism of action of $n-3$ LC-PUFAs, and to promote the use of $n-3$ LC-PUFAs in the prevention and treatment of the MS.

METABOLISM AND BIOLOGICAL EFFECTS OF *n*−3 PUFAs

Fatty acid metabolism and formation of their active metabolites

Animals and humans cannot synthesize PUFAs of the $n-6$ and $n-3$ series, which contain double bonds at C-6 and C-3 respectively, from the methyl end of the molecule. The most plentiful source of $n-3$ LC-PUFAs is marine phytoplankton, a fundamental component of the marine food chain. Precursors for the synthesis of LC-PUFAs of the $n-6$ and $n-3$ series in mammals are linoleic acid ($C_{18:2n-6}$) and ALA respectively. Although linoleic acid and ALA give rise to different metabolites, the enzymes involved in their metabolism are the same. Linoleic acid and ALA compete for the enzyme Δ^6 desaturase, which is required for their further metabolism. Excessive amounts of linoleic acid slows down the formation of EPA and DHA. Even without this inhibitory effect, the synthesis of EPA and DHA from ALA is fairly inefficient. Therefore increased intake of EPA and DHA results in better effects, even if the content of ALA in the diet is high [24]. When EPA is supplemented in the diet of humans, there is an increase in EPA, but no change in DHA, in plasma phospholipids [25].

PUFAs represent the fundamental components of phospholipids in cellular membranes. PUFAs are usually located in the *sn*-2 position, whereas saturated or mono-unsaturated fatty acids are usually bound in the *sn*-1 position of the phospholipid molecules. Fatty acids integrated in these positions reflect the composition of dietary fat. In humans, it may take 4–6 months after the start of DHA supplementation to reach steady-state levels of DHA in the membranes and achieve a full biological effect [12,26]. Many effects of LC-PUFAs depend on the formation of their active metabolites, eicosanoids and other lipid mediators. These molecules are formed after the release of LC-PUFAs from phospholipids by PLA_2 (phospholipase A_2) and act in both autocrine and paracrine manners (Figure 1). They are usually poorly stable and act very rapidly through the use of approx. ten different receptors in immunocompetent cells, platelets, smooth muscle, AT and other tissues (see below). Reasonably often, mediators from the same group have different tissue-dependent biological effects. Synthesis of prostaglandins and thromboxanes depend mostly on the activity of type 1 and 2 COXs (cyclo-oxygenases), for which arachidonic acid ($C_{20:4n-6}$) is a 'better' substrate than EPA, and on the activity of lipoxygenase, whose preferences for arachidonic acid and EPA are opposite compared with COXs [27]. Arachidonic acid and EPA compete for COXs, and both EPA and DHA directly inhibit the activity of this enzyme (Figure 1). A relatively small increase in the $n-3$ LC-PUFA content significantly slows down the synthesis of eicosanoids from arachidonic acid. Eicosanoids derived from $n-3$ LC-PUFAs have, in general, anti-inflammatory effects, whereas the equivalent eicosanoids derived from $n-6$ PUFAs promote inflammation [28]. Moreover, novel families of lipid mediators derived from EPA and DHA, the resolvins and protectins, are potent locally acting agents in the processes of acute inflammation and its resolution. They possess anti-inflammatory pro-resolving effects, as well as providing protection against tissue damage [29].

Probably through the action of these lipid metabolites, $n-3$ LC-PUFAs decrease inflammation, including a low-grade inflammatory response of AT in obesity ([30], and Z. Jilkova, P. Flachs and S. Cinti, unpublished work).

Intracellular regulatory mechanisms affected by *n*−3 LC-PUFAs

The metabolic effects of $n-3$ LC-PUFAs are largely mediated by PPAR (peroxisome-proliferator-activated receptor) transcription factors, with PPARα and PPARδ (PPARβ) being responsible for the lipid-catabolizing

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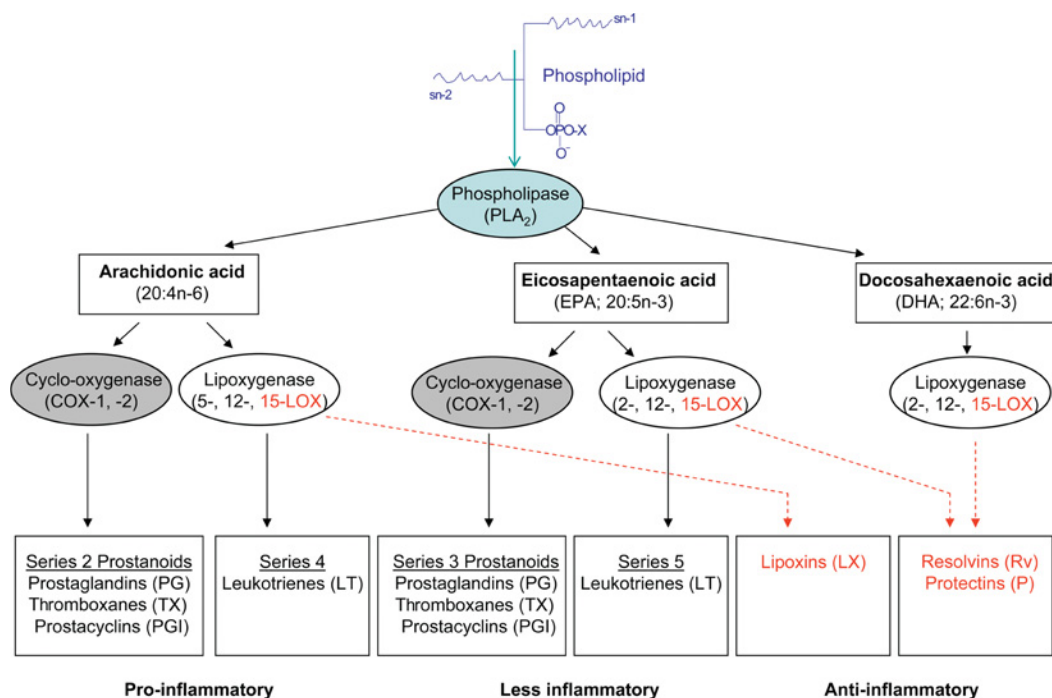

Figure 1 Formation of active metabolites from LC-PUFAs

LC-PUFAs bound to membrane phospholipids at the *sn*-2 position are released by PLA_2 in response to various physiological and pathophysiological stimuli. The released LC-PUFAs are used as substrates for the production of active lipid metabolites acting extracellulary, primarily through G-protein-coupled receptors.

effects of $n-3$ LC-PUFAs. Other transcription factors involved include LXR-α (liver X receptor-α), HNF-4 (hepatic nuclear factor-4) and SREBP-1 (sterol-regulatory-element-binding protein-1) (reviewed in [16]). PUFAs, including DHA, can also function as endogenous ligands of RXR-α (retinoid X receptor-α) while affecting lipid metabolism [31]. Besides acting directly, most of the effects of $n-3$ LC-PUFAs are mediated indirectly through their active metabolites (see above).

Part of the metabolic effects on $n-3$ LC-PUFAs in the liver [32], and possibly also in other tissues, is mediated by the stimulation of AMPK (AMP-activated protein kinase; [33]), a metabolic sensor controlling intracellular metabolic fluxes, namely the partitioning between lipid oxidation and lipogenesis. Phosphorylation of acetyl-CoA carboxylase by AMPK leads to an inhibition of enzyme activity, resulting in a decrease in malonyl-CoA content. Malonyl-CoA is the key lipogenic intermediate, which also inhibits mitochondrial CPT-1 (carnitine palmitoyltransferase-1). Thus AMPK inhibits lipogenesis while stimulating β-oxidation. Moreover, AMPK inhibits gluconeogenesis in the liver and stimulates glucose transport in skeletal muscle. In muscle cells, AMPK is activated by physical activity. Therefore AMPK stimulates the influx of glucose into muscle cells independently of insulin [33,34]. AMPK also induces mitochondrial biogenesis through the activation of NRF-1 (nuclear respiratory factor-1) [35] and the upstream regulatory factor PGC-1α (PPARγ coactivator-1α). This factor links nuclear receptors to the transcriptional programme of mitochondrial biogenesis and oxidative metabolism in both adipocytes and muscle cells [36–38] and to the gluconeogenic programme in the liver [39]. AMPK activation in adipocytes results in the inhibition of fatty acid synthesis and lipolysis [40–43], stimulation of glucose uptake [44] and down-regulation of PPARγ [45]. The physiological role of AMPK in AT remains relatively unexplored (reviewed in [46], and see below). AMPK in the liver is stimulated by metformin [47], a widely used antidiabetic agent. In addition, TZDs (thiazolidinediones), increasingly used for the treatment of patients with diabetes, may stimulate AMPK activity in many tissues, including WAT (white AT) [48], rapidly and independently of PPARγ-mediated gene transcription [49]. The activity of the AMPK regulatory cascade is also modulated by some adipokines: both leptin [50] and adiponectin [51] stimulate AMPK, whereas resistin has the opposite effect [52]. Thus AMPK represents an outstanding therapeutic target and may also be important for the mechanism of action of $n-3$ LC-PUFAs in AT.

Several studies have indicated the involvement of SCD-1 (stearoyl-CoA desaturase-1) in the metabolic effects of PUFAs in the liver and skeletal muscle, where PUFAs down-regulate the *SCD-1* gene [24]. SCD-1 is a central lipogenic enzyme, catalysing the synthesis of mono-unsaturated fatty acids both in the liver [53,54] and skeletal muscle [55]. Using multiple mechanisms, involving the activation of AMPK and suppression

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of acetyl-CoA carboxylase activity, down-regulation of SCD-1 results in increased β-oxidation, enhanced thermogenesis and obesity resistance [54–57]. As SCD-1 is also involved in the formation of ceramides, its down-regulation in skeletal muscle may improve insulin sensitivity [55]. In our experiments, SCD-1 expression was down-regulated by dietary $n-3$ LC-PUFAs in several tissues of mice fed an HF diet, including liver, AT [24] and skeletal muscle (P. Flachs and M. Hensler, unpublished work).

Studies suggest that cannabinoids directly influence both the central nervous system and peripheral tissues, including AT, in which the CB1 receptor (cannabinoid type 1 receptor) is expressed, and that CB1-receptor-knockout mice are resistant to obesity (reviewed in [58]). By analogy to their effects in the brain [59], dietary $n-3$ LC-PUFAs might affect metabolism of peripheral tissues by modulating the formation of the endogenous ligand for the CB1 receptor.

ADIPOSE TISSUE

Biology and secretory functions

Two types of AT found in mammalian organisms, BAT (brown AT) and WAT, differ with respect to their physiological functions, morphology, metabolism and development during ontogeny. BAT is an organ of regulatory non-shivering thermogenesis, mediated by UCP-1 (uncoupling protein-1) in mitochondria, which are abundant in multilocular brown adipocytes [60]. In contrast, large unilocular adipocytes of WAT, filled with triacylglycerols and equipped with a small cytosolic compartment, serve as an energy storage device. During the perinatal period, dynamic recruitment of both BAT and WAT occurs, with important differences among species. Humans are born with substantial amounts of both tissues, with BAT and WAT developing during the last trimester of gestation [61,62]. The amount of BAT declines sharply during the early postnatal period, but it may exist even in the elderly as brown adipocytes interspersed in WAT [63]. Large changes in WAT size and cellularity in response to changes in energy balance represent the typical features of this organ. Both abnormally low or excessively high content of WAT leads to adverse metabolic consequences at the systemic level.

The systemic effects of WAT reflect its ability to function as an endocrine organ, integrating hormonal signals from different parts of the body in response to changes in energy balance and secreting a large number (at least 60–70) of various adipokines acting both at the local (autocrine/paracrine) and systemic (endocrine) level. Through the secretion of adipokines, WAT is involved in the control of energy balance, body temperature, immune response, blood clotting, bone mass, and thyroid and reproductive functions, as well as some other functions. Several adipokines, namely leptin, adiponectin, omentin and visfatin, also exert antidiabetic effects [64–66]. In the peripheral tissues, both leptin [50] and adiponectin [51,67] stimulate AMPK. Part of the metabolic effects of leptin is also attributable to a specific repression of mRNA levels and enzyme activity of SCD-1 (see above). By promoting oxidation of fatty acids in peripheral tissues, leptin and adiponectin protect tissues against the lipotoxic damage, thus permitting a harmless storage of body fat. Obesity is frequently associated with leptin resistance and increased leptinaemia [68,69], while adiponectin concentrations are decreased and closely related to insulin resistance [70]. Moreover, hypertrophic AT secretes various pro-inflammatory cytokines, including TNF-α (tumour necrosis factor-α), IL (interleukin)-6, IL-1 and MCP-1 (monocyte chemoattractant protein-1), and also mediators of the clotting processes, such as PAI-1 (plasminogen activator inhibitor-1) and certain complement factors (for review, see [65]). In fact, systemic low-grade inflammation has been proposed to have an important role in the pathogenesis of obesity-related insulin resistance [71,72]. Studies suggest that (i) TLR4 (Toll-like receptor 4), one of the receptors playing a critical role in the innate immune system, is involved in the activation of the inflammatory pathways in AT by high levels of NEFAs (non-esterified fatty acids) in an obese state [73]; and (ii) AT in lean individuals contains macrophages in an M2-polarized state that may protect adipocytes from inflammation, whereas obesity leads to increased accumulation of macrophages in AT and a shift in the activation state of the macrophages to M1-state, which is inflammatory and contributes to insulin resistance [74,75]. Thus AT of obese individuals contains a large number of macrophages that represent an additional source of pro-inflammatory cytokines [76,77], including TNF-α and other insulin-resistance-promoting adipokines [78,79]. MCP-1 has been identified as a potential factor contributing to macrophage infiltration in AT [80]. Importantly, part of the anti-inflammatory effects of TZDs could result from the activation of M2 macrophages, at least in peripheral blood [81]. This mechanism might also be important for the suppression of a low-grade inflammation in AT [65,78].

AT as a target for the treatment of the MS

Obesity and Type 2 diabetes are associated with insulin resistance, the underlying feature of all components of the MS [1,2]. Surprisingly, despite only a minimal contribution of AT to the whole-body glucose uptake, impairment of glucose transport in adipocytes results in insulin resistance in skeletal muscle and liver [82]. Hypertrophied adipocytes, especially from the intra-abdominal fat depot, are resistant to the antilipolytic effect of insulin [83]. Insulin-resistant adipocytes are also inefficient with regard to the storage of meal-derived NEFAs during postprandial periods [84]. Insufficient 'buffering' capacity of

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adipocytes would lead to ectopic lipid deposition in non-ATs, such as skeletal muscle, resulting in decreased insulin sensitivity [85,86]. The critical role of AT as a metabolic sink for excess NEFAs, which secures normal insulin sensitivity, has been demonstrated further by either transgenic mouse models of lipodystrophy [87] or in genetically obese *ob/ob* mice overexpressing adiponectin [88].

A recent hypothesis suggests that the number and activity of mitochondria within adipocytes contribute to the threshold at which fatty acids are released into the circulation, leading to insulin resistance and Type 2 diabetes [89]. Mitochondrial content was decreased in AT of genetically obese mice [90], whereas this defect was normalized by the treatment with TZDs [91]. A recent study suggests an inverse correlation between mitochondrial oxidative capacity and *in situ* lipogenesis in human WAT and the importance of this link for local control of adiposity in fat depots [92]. In fact, our previous studies indicated that the 'metabolic switch', brought about by the expression of transgenic UCP-1 specifically in WAT of aP2-*Ucp1* transgenic mice, rendered these animals resistant to obesity and glucose intolerance [93,94]. Systematic phenotypic characterization revealed (reviewed in [95]) that the beneficial effect of respiratory uncoupling resulted from a sequence of related events in adipocytes: (i) increased mitochondrial biogenesis [96]; (ii) elevated activity of lipoprotein lipase [97,98]; (iii) induction of fatty acid oxidation and decreased lipogenesis [99]; (iv) lower release of fatty acids into the systemic circulation [98,100]; and (v) lower accumulation of lipids in the liver and skeletal muscle of the transgenic mice (M. Rossmeisl and M. Hensler, unpublished work). We have demonstrated that a key regulatory event stimulated by respiratory uncoupling in adipocytes of transgenic aP2-*Ucp1* mice was the induction of AMPK [101]. Possible involvement of AMPK in the 'metabolic switch' in adipocytes is supported further by the fact that some treatment strategies, including TZDs, adipokines, such as leptin and adiponectin (see above), as well as starvation [102] and physical activity [103], improve various metabolic parameters while activating AT AMPK. Thus AMPK in WAT could be involved in the control of whole-body lipid metabolism, adiposity and insulin sensitivity. Similar to the effect of ectopic UCP-1 in WAT, substances such as leptin [104,105], β_3-adrenergic agonists [106] and bezafibrate [107] are capable of converting adipocytes into fat-burning cells and reducing accumulation of body fat in rodents [104,107]. A similar effect was also observed in our experiments with $n-3$ LC-PUFAs, even without induction of UCPs in adipocytes ([24]; and see below), and could be possibly caused by a reduction in CB1 receptor signalling in WAT ([58,59]; see above).

The findings mentioned above, as well as other evidence (reviewed in [108]), strongly support the notion that energy metabolism of WAT and its secretory functions are central to the pathophysiology of the MS. Therefore AT represents a suitable target for the prevention and treatment of the MS.

ADIPOSE TISSUE AS A TARGET FOR $n-3$ LC-PUFAs

Accumulation and storage of $n-3$ LC-PUFAs in WAT

WAT represents an important player involved in the effect of PUFAs, due to its storage capacity for triacylglycerols, the most concentrated form of fatty acids including PUFAs. In this respect, AT of nursing mothers serves as a buffer for $n-3$ LC-PUFAs, thus preventing large fluctuations in their concentration in breast milk [109]. In addition, in adults, WAT represents the main storage site of PUFAs, including $n-3$ LC-PUFAs, as it represents approx. 15–25 % of body weight in lean individuals (this percentage can increase by more than 50 % in cases of morbidly obese patients), whereas approx. 70 % of AT mass is formed by lipids [110]. Similar to liver and skeletal muscle, the fatty acid composition in AT triacylglycerols approximately corresponds to the composition of fatty acids in the diet [111]. Importantly, in addition to the storage of DHA in AT, a substantial portion of DHA is contained in brain phospholipids. DHA constitutes almost 17 % of the total fatty acids in the brain [112].

AT is also a major reservoir for many different lipophilic contaminants [110]. Most of them are POPs (persistent organic pollutants), such as polychlorinated biphenyls, dioxins and hexachlorobenzenes (reviewed in [113]), which are also accumulated in higher trophic levels of the food chain. Thus food, especially fatty fish, meat and milk products, is the main source of human exposure to POPs. In particular, pregnant women must be advised to choose contaminant-free sea food. On the other hand, their daily intake of $n-3$ LC-PUFAs should be similar to the rest of the population [for the recommendations concerning dietary intake of $n-3$ LC-PUFAs in different countries, see the ISSFAL (International Society for the Study of Fatty Acids and Lipids) website at http://www.issfal.org.uk/]. Thus fish oil concentrates, in which environmental pollutants have been removed during their manufacturing, represent a suitable alternative for individuals who could benefit from an increased intake of $n-3$ LC-PUFAs.

Prevention of AT growth and proliferation of adipocytes by $n-3$ LC-PUFAs

AT mass can increase either by hypertrophy or hyperplasia of fat cells. During differentiation, pluripotent stem cell precursors give rise to multipotent mesenchymal precursor cells, preadipocytes and differentiated mature adipocytes [114]. The key role in the differentiation process is played by transcription factors from the PPAR

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(see below) and C/EBP (CCAAT/enhancer-binding protein) families [114]. Our results, obtained in adult C57BL/6 mice fed an HF diet (corn oil as a major lipid component), have demonstrated that substitution of only 9 % of dietary lipids in the HF diet by EPA/DHA (i.e. the replacement of 15 % of the lipids by the EPA/DHA concentrate EPAX 1050 TG) prevented fat accumulation with a preferential reduction in abdominal fat depots [24,115]. Using $n-3$ LC-PUFA concentrates which differed in the EPA to DHA ratio (EPAX 1050 TG and EPAX 4510 TG respectively), we have shown that the protective effect of $n-3$ LC-PUFAs on AT accumulation was stronger in the case of DHA compared with EPA and resulted, in part, from the inhibition of fat cell proliferation [115]. In experiments using cell cultures, DHA inhibited adipocyte differentiation and induced apoptosis in post-confluent preadipocytes [116]. DHA also induces apoptosis in several models of cancer [117].

The effects of $n-3$ LC-PUFAs on proliferation and maturation of adipose cells may be caused by altering phospholipid composition of the cellular membrane with consequent changes in eicosanoid biosynthesis (Figure 2). Preadipocytes, as well as adipocytes, produce significant amounts of prostaglandins (PGE_2, $PGF_{2\alpha}$ and PGD_2) and prostacyclins (PGI_2), i.e. eicosanoids of the '2-series' derived from arachidonic acid (see Figure 1). Feeding diets rich in $n-3$ PUFAs results in a decreased arachidonic acid content in membrane phospholipids of AT [118], while slowing down the synthesis of eicosanoids. For instance, decreased formation of PGD_2 and its 15-deoxy-J_2 derivate, known PPARγ ligands and inducers of adipogenesis [114], would be compatible with the effect of DHA on the differentiation of adipocytes *in vitro* (see above). PGI_2 released by differentiated adipocytes is able to induce preadipocytes to differentiate [119]. *In vivo*, this paracrine effect may represent a crucial signal in the hyperplastic development of AT known to occur once adipocytes reach their maximal size [114].

The antiproliferative effect of $n-3$ LC-PUFAs may be involved in the decreased adiposity of pups born to rat or mouse dams that were fed diets supplemented by $n-3$ LC PUFAs [120] or ALA [15] during gestation and suckling. It has been hypothesized that LC-PUFAs are also involved in the anti-obesity [121] and antidiabetic effects [122] of breastfeeding. A relatively high intake of $n-6$ compared with $n-3$ PUFAs during the pregnancy, suckling period and early infancy could lead to childhood obesity. This may be of particular importance for modern human society facing an increased dietary $n-6/n-3$ PUFA ratio [14,15,120]. However, presumptions about the role of the dietary $n-6/n-3$ PUFA ratio in determining AT development are derived exclusively from experiments in mice [15], and further studies are required before a definite conclusion can be made [123].

Modulation of AT metabolism by $n-3$ LC-PUFAs

EPA, DHA and some eicosanoids modulate gene expression through a variety of transcription factors and their effects are tissue-specific (see above and Figure 2). One of the important targets, PPARγ, binds not only lipid molecules, but also TZDs [85,124,125]. After ligand binding, PPARγ stimulates expression of genes engaged in differentiation of fat cells, namely genes encoding fatty acid transporters and lipogenic genes. In addition, other members of the PPAR family, PPARα and PPARδ, can be activated in adipocytes, resulting in the stimulation of fatty acid oxidation in mitochondria and peroxisomes [16,126]. The AMPK and SCD-1 regulatory cascades may be also involved in the effects of $n-3$ LC-PUFAs on AT metabolism (see above). Our experiments in mice have shown a marked down-regulation of the *Scd-1* gene in WAT by $n-3$ LC-PUFAs admixed to an HF diet [24], suggesting the involvement of SCD-1 in the induction of lipid catabolism in AT (see below).

We have found [24] that a more pronounced effect of $n-3$ LC-PUFAs in the prevention of obesity induced by an HF diet in mice, as compared with their precursor ALA, may be mediated by the induction of mitochondria in WAT. The replacement of 15 % of the dietary lipids in the HF diet with an EPA/DHA concentrate (6 % EPA and 51 % DHA; EPAX 1050 TG) resulted in the up-regulation of genes for mitochondrial proteins predominantly in epididymal fat [24]. The effect of EPA/DHA in abdominal fat was associated with a 3-fold increase in the expression of genes for regulatory factors for mitochondrial biogenesis and oxidative metabolism, namely PGC-1α and NRF-1 respectively. In contrast with WAT, no changes in the expression of mitochondrial genes were observed in either the liver [24] or skeletal muscle (P. Flachs, M. Hensler and J. Kopecky, unpublished work). The expression of genes for CPT-1 and fatty acid oxidation were increased in epididymal, but not in subcutaneous, fat. In the former fat depot, palmitate oxidation was increased, whereas lipogenesis was depressed. Moreover, gene expression of PGC-1α and NRF-1 were also stimulated by $n-3$ LC-PUFAs in 3T3-L1 adipocytes differentiated in cell culture [24]. Surprisingly, no induction of UCP-1 by dietary $n-3$ LC-PUFAs was detected either in BAT or WAT, and no increase of UCP-2 expression in WAT was observed [24]. Thus the anti-obesity effect of $n-3$ LC-PUFAs may result, at least in part, from increased lipid catabolism and depression of lipogenesis in adipocytes, independent of respiratory uncoupling (Figure 2). Similar metabolic changes in AT ('metabolic switch'), such as the up-regulation of genes for CPT-1 [105,107] and PGC-1α [104], could be also induced by other treatments, including leptin, together with the activation of AMPK in adipocytes (see above). In addition, CNTF (ciliary neurotrophic

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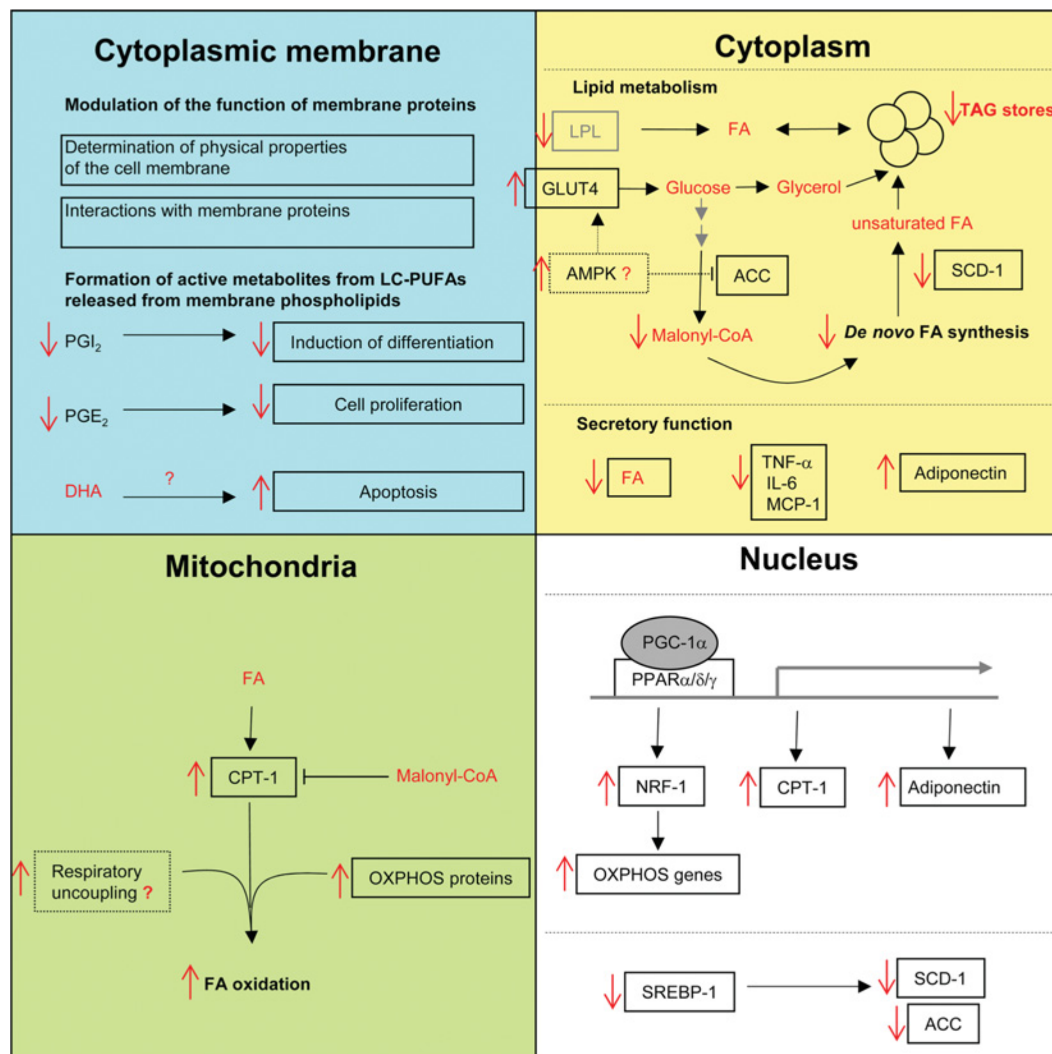

Figure 2 Effects of $n-3$ LC-PUFAs in adipocytes

Overview of the actions of $n-3$ LC-PUFAs on gene expression, lipid metabolism and secretory function of adipocytes. ACC, acetyl-CoA carboxylase; FA, fatty acids; LPL, lipoprotein lipase; OXPHOS, oxidative phosphorylation; TAG, triacylglycerol.

factor) reversed obesity in mice by re-programming AT to promote mitochondrial biogenesis, enhancing oxidative capacity and reducing lipogenic capacity while increasing lipid oxidation and AMPK activity in adipocytes [127]. Whether AMPK is involved also in the effect of $n-3$ LC-PUFAs in WAT remains to be established (Figure 2). A combined treatment by the EPA/DHA concentrate (EPAX 1050 TG) admixed to the HF diet and mild caloric restriction has been suggested to exert additivity both in the reduction of adiposity and in the stimulation of mitochondrial biogenesis in AT (P. Flachs, unpublished work). Caloric restriction has been shown previously to induce mitochondrial biogenesis in several tissues via the activation of the NO signalling pathway [128]. Production of NO in WAT, namely in the endothelial cells of its blood vessels, could be stimulated by adiponectin (see [129] and below).

In accordance with the increased catabolism of fatty acids in adipocytes, basal lipolysis, which is significantly increased in obese and insulin-resistant rats fed a sucrose-rich diet, was also normalized by $n-3$ LC-PUFAs admixed with the diet [9]. This effect probably depends on improved insulin sensitivity of AT and restoration of the antilipolytic effect of insulin. In contrast with the effect of DHA in obese animals, DHA promoted lipolysis in adipocytes differentiated in cell culture [116]. This paradoxical *in vitro* effect could be explained by the suppressed formation of PGE_2 in the presence of DHA [118], as PGE_2 negatively modulates lipolysis via its specific receptor EP4 [27]. It has also been shown that $n-3$ LC-PUFAs decreased the activity of lipoprotein lipase in abdominal AT of rats while ameliorating obesity induced by a high-carbohydrate diet [9], in accordance with reduced lipoprotein lipase gene expression in

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response to n−3 LC-PUFA in rats fed an HF diet [130]. Therefore increased fatty acid oxidation in fat cells due to n−3 LC-PUFA feeding possibly contributes to their anti-obesity effect and to the shrinkage of adipocytes, but it is not associated with increased fatty acid uptake into AT. These experiments suggest that decreased lipogenesis and increased lipid oxidation in the liver and skeletal muscle, rather than increased uptake of fatty acids in AT, are of primary importance in the hypolipidaemic effect of n−3 LC-PUFAs [9,16].

The induction of mitochondrial biogenesis in murine AT by n−3 LC-PUFAs was detected in mature adipocytes, released from AT by collagenase digestion [24], and was associated with the up-regulation of PPARγ in these cells (P. Flachs, unpublished work). In addition, TZDs stimulate the formation of mitochondria in AT [91,131]. However, in contrast with EPA/DHA, TZDs induce AT growth, especially in the abdomen [132,133], indicating multiple and distinct mechanisms of action in the effects of n−3 LC-PUFAs and TZDs. Treatment with n−3 LC-PUFAs or the TZD rosiglitazone [30,115,134] resulted in a significant decrease in the size of mature adipocytes in epididymal fat compared with those in the control HF-fed animals, and this effect was enhanced further by the combination treatment with n−3 LC-PUFAs and rosiglitazone (O. Kuda, T. Jelenik, M. Rossmeisl and J. Kopecky, unpublished work). Even in human subjects with diabetes, n−3 LC-PUFAs decreased the size of fat cells [135]. The formation of small adipocytes may result from proliferation of preadipocytes [30,115,134] and/or from shrinkage of existing mature adipocytes due to catabolism of their triacylglycerol stores [16,24,91,136]. Small adipocytes could be involved in the antidiabetic action of the above treatments. Compared with large adipocytes, small cells are more insulin-sensitive and less lipolytic, release less inflammatory cytokines (reviewed in [133]) and secrete more adiponectin [137]. The small cells could also serve as a 'buffer' for lipids and protect tissues against lipotoxicity (see above, and [85,86]). In fat cells, n−3 LC-PUFAs also affect the expression of the GLUT-4 (glucose transporter-4) gene [115] and, hence, could influence glucose uptake into the cells [9]. In rodents, both the expression of GLUT-4 and glucose uptake in adipocytes is inhibited by an HF diet in parallel with the induction of insulin resistance. Admixing EPA/DHA to the HF diet protected animals against the down-regulation of GLUT-4 [19].

Modulation of secretion of adipokines by n−3 LC-PUFAs

That adiponectin may be induced in response to dietary n−3 LC-PUFAs was demonstrated for the first time in rats fed a sucrose-rich (approx. 60 g of sucrose/100 g) diet [10]. Long-term (9 months) feeding with this diet resulted in a decrease in plasma adiponectin levels. Shifting the source of dietary fat from corn oil to fish oil (i.e. replacing 7 g out of 8 g of oil/100 g diet) during the last 2 months of experimental feeding increased plasma adiponectin over the controls and normalized leptin levels, while reversing whole-body insulin resistance, dyslipidaemia and AT hyperplasia [10]. In our experiments on mice fed a corn-oil-based HF diet (35 g of fat/100 g of diet), the replacement of 15 % of dietary lipids by an EPA/DHA concentrate prevented the development of obesity and insulin resistance (see above), while plasma adiponectin levels as well as adiponectin release from epididymal AT were increased [138]. In a similar study on mice fed a safflower-oil-based HF diet (27 g of fat/100 g diet), performed by Shulman and co-workers [139], n−3 LC-PUFAs also induced adiponectin, and this induction was completely blocked by a PPARγ inhibitor [139]. In contrast, the effect of n−3 LC-PUFAs on adiponectin secretion was retained in PPARα-null mice [139]. Both studies showed an induction of adiponectin in epididymal, but not in subcutaneous, fat [138,139]; accordingly, abdominal fat is a more important producer of adiponectin than subcutaneous fat [138,140]). Our study [138] also demonstrated that fully differentiated adipocytes represented the main source of adiponectin in mice treated with n−3 LC-PUFAs. In contrast with the study by Shulman and co-workers [139], our experiments indicated that the induction of adiponectin gene expression by orally administered n−3 LC-PUFAs required more than 24 h, whereas the absence of the induction during the first 24 h was independent of the EPA/DHA ratio in the diet, ranging between 0.4 and 4.0 (P. Flachs and M. Hensler, unpublished work). In addition, in contrast with the results by Shulman and co-workers [139], in which various n−3 PUFAs failed to stimulate adiponectin mRNA expression in 3T3-L1 adipocytes, a recent report [141] documented the induction of the adiponectin gene by n−3 LC-PUFAs in this adipocyte cell line, with DHA having the most pronounced effect. Thus n−3 LC-PUFAs represent naturally occurring inducers of adiponectin, acting perhaps via PPARγ upon the adiponectin gene promoter ([142], and see Figure 2).

The induction of mitochondrial biogenesis in adipocytes by n−3 LC-PUFAs (see above) could be directly involved in the stimulation of adiponectin secretion [143]. In turn, adiponectin could induce NO formation in the endothelium of blood vessels in AT [129] and, hence, activate the regulatory pathway of mitochondrial biogenesis (see above, and [128]) as well as local vasodilation [129]. NO is also involved in a positive regulatory loop controlling adiponectin release [144]. The mechanisms above may be closely linked to defective NO bioavailability described in patients with the MS [145]. In contrast with adiponectin, TNF-α down-regulates the activity of the NO signalling pathway, while depressing mitochondrial biogenesis in fat and muscle of obese rodents [146]. Adiponectin is also induced in response to a CB1 receptor antagonist [147], i.e. under the conditions that promote

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obesity resistance in mice [58]. Accordingly, the formation of an endogenous ligand for the CB1 receptor might be reduced by *n*−3 LC-PUFA treatment (see above, and [59]). Further studies are required to clarify the role of *n*−3 LC-PUFAs in the integrated control of secretory functions and mitochondrial biogenesis in adipocytes.

A clinical study [12] has demonstrated the induction of plasma adiponectin in response to a daily intake of 1.3 g of EPA and 2.9 g of DHA (administered as EPAX 2050 TG) in overweight patients treated simultaneously by a weight-loss programme. The effect was stronger after 6 months compared with 3 months of treatment [12]; however, an appropriate control group without the weight-loss programme was not included in that study. In our experiments, the induction of adiponectin in mice fed an HF diet was not affected by a 30 % caloric restriction [138]. In accordance with the *in vitro* results (see above), the intake of DHA, rather than EPA, also appeared to be important for the induction of adiponectin in humans, where a relatively slow increase in plasma adiponectin levels correlated with the time course of the increase in DHA concentrations in AT [12]. The induction of adiponectin could be of great importance for the beneficial effect of *n*−3 LC-PUFAs on systemic insulin sensitivity, reflecting their paracrine anti-inflammatory effects in AT (see below).

With regard to the effect of *n*−3 LC-PUFAs on other adipokines besides adiponectin, experimental evidence is much weaker and conflicting. Feeding rats a sucrose-rich diet resulted in a decrease in plasma leptin levels, which was prevented by dietary *n*−3 LC-PUFAs [10], whereas feeding mice an HF diet resulted in increased plasma leptin levels that were not affected by lower doses [138,139], but were depressed by a higher dose of *n*−3 LC-PUFAs in the diet [139]. Early postnatal development may represent a critical period for the modulation of leptin gene expression by PUFAs [120,148].

In addition, the secretion of other adipokines could be affected by *n*−3 LC-PUFA administration, as these fatty acids are capable of reducing the low-grade inflammation of AT associated with obesity. This was demonstrated in obese diabetic *db/db* mice [30] as well as in inbred C57BL/6 mice fed an HF diet (Z. Jilkova and P. Flachs, unpublished work). In both studies, a partial replacement of dietary lipids by *n*−3-LC-PUFAs prevented macrophage infiltration of AT and down-regulated inflammatory gene expression. In *db/db* mice, the anti-inflammatory effect of *n*−3 LC-PUFAs was not associated with a decrease in adiposity, leading to a separation of their anti-obesity effects from anti-inflammatory action [30]. In both studies, *n*−3 LC-PUFAs induced adiponectin, similar to the effect of TZDs, which are also capable of lowering resistin and leptin secretion from fat cells while counteracting AT inflammation [65,136,149–152]. Our studies in C57BL/6 mice revealed additivity in the effects of TZDs and *n*−3 LC-PUFAs on the reduction of AT inflammation and induction of adiponectin (O. Kuda, Z. Jilkova and P. Flachs, unpublished work). However, no effect of *n*−3 LC-PUFAs on plasma levels of resistin [139], IL-6 or MCP-1 levels in the obese diabetic *db/db* mice was observed [30]. Only some human studies have shown reductions in pro-inflammatory cytokines after dietary supplementation with fish oil [12,28]. Therefore, with respect to a possible modulation of inflammatory cytokine secretion by *n*−3 LC-PUFAs, especially from AT, further studies are required. New insights into the mechanism of the control of adiposity by *n*−3 LC-PUFAs might be obtained. Perhaps, through the induction of adiponectin (see above) and consequent down-regulation of TNF-α, *n*−3 LC-PUFAs could also decrease the local production of cortisol in AT, and thus interfere with the development of obesity (for references, see [153]). Some conflicting findings could most probably be explained by the use of different *n*−3 LC-PUFA products. Consequently, the effect of *n*−3 LC-PUFAs in the production of pro-inflammatory mediators in macrophages may depend on the ratio of EPA to DHA [154]. Future studies should verify whether *n*−3 LC-PUFAs can activate M2 macrophages in AT, i.e. activate the cells capable of the secretion of anti-inflammatory cytokines, similar to the effect of TZDs on macrophages in peripheral blood (see above, and [81]).

FUTURE DIRECTIONS

The effectiveness of *n*−3 LC-PUFAs in the modulation of gene expression, metabolism and other biological properties of the organism depends on the duration of *n*−3 LC-PUFA administration and on the composition of dietary lipids. In the case of *n*−3 LC-PUFA concentrates, the effectiveness may depend on the ratio of EPA to DHA, as well as on the formulation of the concentrate. Thus, although the hypolipidaemic effect of *n*−3 LC-PUFAs probably depends mainly on EPA or its metabolites [17], other beneficial effects, including the induction of adiponectin [12,139] or reductions in body weight, adiposity and AT cellularity [23,115], may be more strongly associated with DHA or its active metabolites. Bioavailability of *n*−3 LC-PUFAs might be affected by the structural form of dietary lipids, i.e. whether EPA and DHA are administered as triacylglycerols, ethyl esters or phospholipids. Triacylglycerols are quantitatively the most important lipid component in the human diet and they are generally very well absorbed in the intestine (> 95 % efficiency). Ethyl esters do not appear to be an efficient form [155], and long-term feeding is necessary to achieve a similar level of tissue incorporation of EPA and DHA compared with triacylglycerol. *n*−3 LC-PUFAs introduced in the form of phospholipids appear to be very efficient in increasing LC-PUFA levels in brain and also in other organs [156]. Further investigations are needed to clarify this matter.

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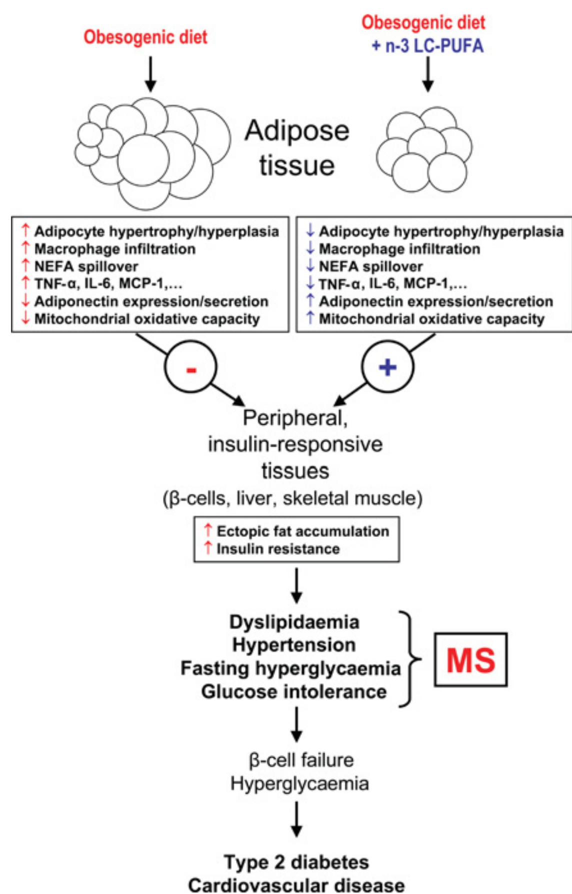

Figure 3 Central role of WAT in the development of systemic insulin resistance and protective effects of dietary $n-3$ LC-PUFA supplementation

A general overview of beneficial effects of $n-3$ LC-PUFAs. The conclusions are drawn mainly from experiments in animal models of diet-induced obesity, in which either a lipogenic sucrose-rich diet or an HF diet was used.

Relatively recently, dual-PPAR agonists targeting two distinct families of PPARs (PPAR-α and PPAR-γ) have been introduced as a novel strategy for the treatment of abnormalities in lipid and glucose metabolism associated with the MS and Type 2 diabetes. Despite concerns about their safety [157], this class of agents have a large therapeutic potential [158]. Likewise, attempts have been made to synthesize chemical derivatives of DHA with the properties of dual-PPAR agonists [159,160]. The first results suggested that these compounds could lower blood glucose in rat and mouse models of diabetes [160]; however, it is too early to conclude whether this strategy will also be successful in humans.

Providing that the metabolism of AT and secretion of adipokines, as well as the inflammatory status of AT, represent important targets for the prevention and treatment of the MS (Figure 3), it should be established whether the anti-obesity and antidiabetic effects of $n-3$ LC-PUFAs, and especially of the DHA-rich concentrates, could be improved by combining dietary $n-3$ LC-PUFAs with other treatments, thus affecting AT by multiple mechanisms. For instance, combination treatments of $n-3$ LC-PUFAs and caloric restriction or $n-3$ LC-PUFAs and antidiabetic/anti-obesity drugs should be explored.

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