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n-3 PUFA: bioavailability and modulation of adipose tissue function

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Adipose tissue has a key role in the development of metabolic syndrome (MS), which includes obesity, type 2 diabetes, dyslipidaemia, hypertension and other disorders. Systemic insulin resistance represents a major factor contributing to the development of MS in obesity. The resistance is precipitated by impaired adipose tissue glucose and lipid metabolism, linked to a low-grade inflammation of adipose tissue and secretion of pro-inflammatory adipokines. Development of MS could be delayed by lifestyle modifications, while both dietary and pharmacological interventions are required for the successful therapy of MS. The *n*-3 long-chain (LC) PUFA, EPA and DHA, which are abundant in marine fish, act as hypolipidaemic factors, reduce cardiac events and decrease the progression of atherosclerosis. Thus, *n*-3 LC PUFA represent healthy constituents of diets for patients with MS. In rodents *n*-3 LC PUFA prevent the development of obesity and impaired glucose tolerance. The effects of *n*-3 LC PUFA are mediated transcriptionally by AMP-activated protein kinase and by other mechanisms. *n*-3 LC PUFA activate a metabolic switch toward lipid catabolism and suppression of lipogenesis, i.e. in the liver, adipose tissue and small intestine. This metabolic switch improves dyslipidaemia and reduces ectopic deposition of lipids, resulting in improved insulin signalling. Despite a relatively low accumulation of *n*-3 LC PUFA in adipose tissue lipids, adipose tissue is specifically linked to the beneficial effects of *n*-3 LC PUFA, as indicated by (1) the prevention of adipose tissue hyperplasia and hypertrophy, (2) the induction of mitochondrial biogenesis in adipocytes, (3) the induction of adiponectin and (4) the amelioration of adipose tissue inflammation by *n*-3 LC PUFA.

Metabolic syndrome: EPA: DHA: Fat

Many studies indicate the key role of hypertrophic adipose tissue in the development of various morbidities in obese individuals, including type 2 diabetes, dyslipidaemia and hypertension, i.e. the major components of metabolic syndrome (MS). Insulin resistance, the central defect underlying the MS, most probably results from increased accumulation of lipids in the peripheral tissues (lipotoxicity) as a result of enhanced release of fatty acids from hypertrophic fat cells[1,2]. In addition to other lifestyle interventions[3,4], adjustment of the quality of dietary lipids is also important for the prevention and treatment of MS. In particular, long-chain (LC) PUFA of the *n*-3 series, DHA (22: 6*n*-3) and EPA (20: 5*n*-3), which are abundant in marine fish, lower TAG while increasing HDL-cholesterol levels in plasma, prevent the development of heart disease and exert anti-inflammatory properties in human

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subjects[5–7]. Studies in rats and mice fed a high-fat or lipogenic sucrose-rich diet have shown that *n*-3 LC PUFA counteract the development of both obesity and insulin resistance[8–13]. Also, in human subjects *n*-3 LC PUFA could reduce fat accumulation and improve glucose metabolism[14–16]. Although *n*-3 LC PUFA appear to have little effect on glycaemic control in patients with type 2 diabetes, these fatty acids are considered to be healthy dietary constituents for this type of patients as a result of the beneficial effect on the plasma lipid profile[17,18].

Animals and human subjects cannot synthesize PUFA, which contain double bonds at C-6 and C-3 from the methyl end of the molecule. Precursors for the synthesis of *n*-6 and *n*-3 LC PUFA are linoleic acid (18: 2 *n*-6) and α-linolenic acid (ALA; 18: 3 *n*-3) respectively. The conversion of ALA to EPA and DHA occurs primarily in the liver. Linoleic acid and ALA compete for the enzyme Δ6 desaturase, which is required for their further metabolism. Thus, an excessive amount of linoleic acid slows down the formation of EPA and DHA. Even without this inhibitory effect, the synthesis of EPA and DHA from ALA, the major proportion of which is rapidly oxidized, is quite inefficient. Thus, (1) supplementation of diets with *n*-3 LC PUFA results in a much higher increase in the plasma and tissue EPA and DHA content when compared with supplementation of the diet with ALA (for reviews, see Arterburn *et al.*[19] and Brenna *et al.*[20]) and (2) the effects of *n*-3 LC PUFA also depend on dietary *n*-6 PUFA:*n*-3 PUFA, which was lower in the diet of ancient hunter–gatherers compared with that of modern humans and is still increasing in affluent societies[21,22]. EPA, DHA, ALA and arachidonic acid (20: 4*n*-6) are incorporated into cellular membranes through their binding to the sn-2 position in the phospholipid molecule. These fatty acids thus influence the fluidity of plasma membranes and the function of membrane proteins. Moreover, the competition for binding to phospholipids also affects the availability of *n*-3 LC PUFA as substrates for cyclooxygenases and lipoxygenases after their release by the action of phospholipases, as well as the formation of their active metabolites, eicosanoids and other lipid mediators[19,23,24]. In general, eicosanoids derived from *n*-3 LC PUFA have anti-inflammatory effects, while the equivalent eicosanoids derived from *n*-6 PUFA promote inflammation[25]. Lipid mediators derived from EPA and DHA, resolvins and protectins, are potent locally-acting agents in processes of acute inflammation and its resolution. They possess anti-inflammatory effects, as well as providing protection against tissue damage[26].

The biological effects of *n*-3 LC PUFA and their metabolites are largely mediated by PPAR, with PPARα and PPARδ (-β) representing the main targets[27–29]. However, PPARγ, liver X receptor-α, hepatic nuclear factor-4, sterol regulatory element-binding protein-1 and NF-κB[7] are also involved[30–32]. The hypolipidaemic and anti-obesity effects of *n*-3 LC PUFA probably depend on the *in situ* suppression of lipogenesis and increase in fatty acid oxidation in several tissues including liver, intestine, and adipose tissue[12,31,33,34]. This metabolic switch may reduce the accumulation of toxic fatty acid derivatives, while protecting the insulin signalling in liver and muscle[9,13,24,30,35]. Part of the metabolic effects of *n*-3 LC

PUFA in the liver[36], and possibly also in other tissues[24,37] (also, see later), is mediated by the stimulation of AMP-activated protein kinase (AMPK), a metabolic sensor controlling intracellular metabolic fluxes, i.e. the partitioning between lipid oxidation and lipogenesis (for review, see Flachs *et al.*[23] and Carling[38]). Thus, *n*-3 LC PUFA by multiple mechanisms of action modulate the functions of all major tissues involved in the development of MS, i.e. the liver, adipose tissue and skeletal muscle[23].

The aim of the present report is to characterize adipose tissue as a target for *n*-3 LC PUFA in the prevention and treatment of pathological conditions associated with MS. Despite a relatively small increase in *n*-3 LC PUFA concentrations in adipose tissue lipids in response to dietary intake of these fatty acids, adipose tissue is specifically linked to the beneficial effects of *n*-3 LC PUFA on health. This relationship is indicated by (1) the prevention of adipose tissue hyperplasia and hypertrophy, (2) the induction of mitochondrial biogenesis in adipocytes, (3) the induction of adiponectin secretion and (4) the amelioration of adipose tissue inflammation by *n*-3 LC PUFA. The present report represents an extension of a review published recently[23], as it contains new results relating to bioavailability (i.e. incorporation of EPA and DHA administered in the diet into plasma lipids) and tissue accumulation of *n*-3 LC PUFA, as well as describing the effects of *n*-3 LC PUFA on AMPK activity and low-grade inflammation of adipose tissue. All the experiments described in the present report were performed using adult male C57BL/6 mice fed a maize oil-based high-fat (cHF; approximately 35 % (w/w) fat) diet, free of DHA and EPA and containing a low level of ALA (approximately 2–4 % (w/w) of total fatty acids [11,13]), which induces the MS phenotype in the mice within several weeks of feeding. The effects of *n*-3 LC PUFA were studied using a concentrate (w/w; approximately 46 % DHA and 14 % EPA; EPAX 1050 TG; EPAX AS, Aalesund, Norway) to replace 5, 15 or 44 % (w/w) dietary fat in the cHF diet[11].

Bioavailability of *n*-3 long-chain PUFA and capacity of adipose tissue for *n*-3 long-chain PUFA storage

Despite the low rate of conversion of ALA to EPA and DHA, both animal and human studies indicate that (1) diets containing only ALA as a source of *n*-3 fatty acids support the formation of limited amounts of both EPA and DHA, resulting in a relatively low plasma and tissue content of these fatty acids and (2) increased supply of dietary ALA results in increases in ALA and EPA content in plasma and tissues, but has no effect on plasma DHA concentration[19,39]. Dietary intake of fish oil or concentrates containing both EPA and DHA results in increased incorporation of both fatty acids into plasma lipids, a measure of the bioavailability of the administered compounds. In human subjects steady-state levels of *n*-3 LC PUFA in total plasma lipids are reached within approximately 1 month, while incorporation of *n*-3 LC PUFA into erythrocytes (and presumably tissues) exhibits slower kinetics[19,20]. The experiments with mice have investigated both the bioavailability (Fig. 1(A and B)) and the
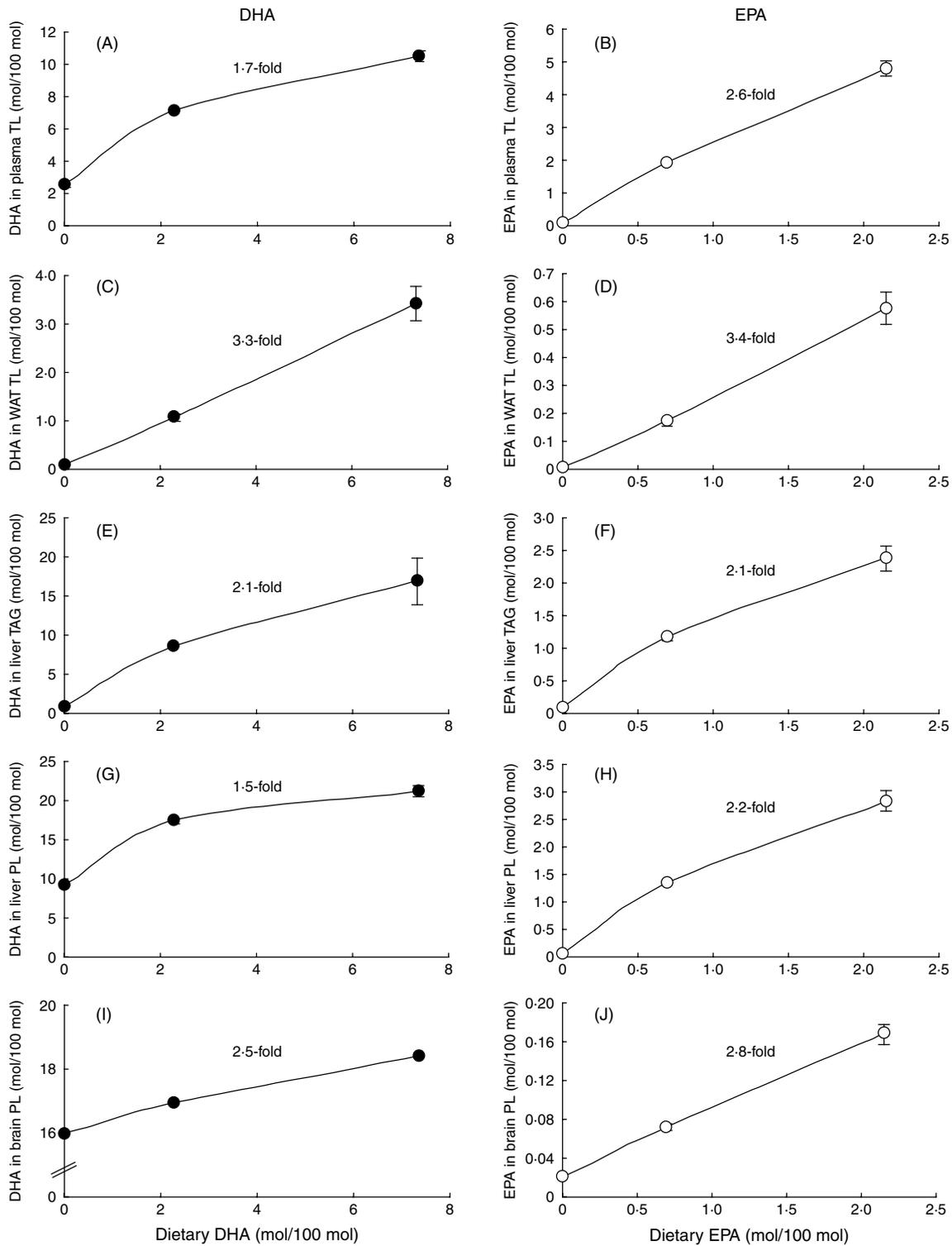
Fig. 1. Bioavailability of DHA and EPA and incorporation of these fatty acids into total lipids (TL) in white adipose tissue (C, D), liver TAG (E, F) and phospholipid (PL; G, H) fractions and brain PL (I, J). At 3 months of age mice were placed on a maize-based high-fat (cHF) diet or the cHF diet with 5% (w/w; D5) or 15% (w/w; D15) of its lipid replaced by the *n*-3 long-chain PUFA concentrate EPAX 1050 TG (EPAX AS, Aalesund, Norway). After 9 weeks of treatment the mice were killed and plasma and tissues collected for analysis of DHA (●) and EPA (○) content. (A, C, E, G, I), The relationship between measured dietary DHA concentration (*n* 3) and measured DHA content (*n* 4–7) in plasma and various tissues; (B, D, F, H, J), corresponding results for EPA. (A, B), DHA and EPA respectively in plasma TL. The fold increases in the corresponding fatty acid as a result of actual measured 3·1-fold increase in DHA or EPA concentration in dietary lipids (from 5% (w/w) to 15% (w/w) in D5 and D15 diet respectively; basal values measured in cHF-fed mice were subtracted) are also shown. Values are means with their standard errors represented by vertical bars.

incorporation of EPA and DHA into total adipose tissue lipids (Fig. 1(C and D)), liver TAG (Fig. 1(E and F)) and phospholipid (Fig. 1(G and H)) fractions and brain phospholipids (Fig. 1(I and J)). In mice fed cHF at the 5 or 15% (w/w) level of substitution for 9 weeks both the DHA and EPA contents of plasma and tissue lipids are increased in response to increasing doses of *n*-3 LC PUFA in the diets. Plasma fatty acid levels remain similar between 2 and 9 weeks of the treatment (not shown), indicating relatively fast kinetics in relation to the equilibration of the total lipid pool in this compartment. The DHA:EPA in plasma is similar to that in the diet; however, a relatively high accumulation of DHA is observed in the tissue lipids, with the most dramatic difference between accumulation of DHA and EPA observed in brain phospholipid fraction (Fig. 1(I and J))[40]. These differences indicate different metabolism of DHA and EPA, as well as specific transport mechanisms for these fatty acids in various body compartments[19,41]. EPA accumulates proportionally to its dietary content, except for liver TAG (Fig. 1(F)) and phospholipid (Fig. 1(H)) fractions, suggesting saturation at higher dietary intakes of EPA. Except for a linear dose response in total adipose tissue lipids (Fig. 1(C)) and brain phospholipids (when the values measured in the cHF-mice are subtracted; Fig. 1(I)), DHA incorporation in total plasma lipids (Fig. 1(A)), liver TAG (Fig. 1(E)) and phospholipid (Fig. 1(G)) fractions is saturable in relation to the dietary content of DHA. Importantly, even in the absence of any EPA or DHA in the cHF diet, substantial amounts of DHA are detected in liver (Fig. 1(G)) and especially in the brain (Fig. 1(I)) phospholipids, indicating quite efficient formation of EPA from ALA contained in the diet and preferential deposition of DHA in these tissues. The linear correlation between the accumulation of both EPA and DHA in total adipose tissue lipids and the dietary content of these fatty acids could reflect different molar concentrations of *n*-3 LC PUFA in adipose tissue lipids as compared with, for example, liver TAG, which are several-fold lower in the case of adipose tissue[41]. However, despite a relatively low specific content of *n*-3 LC PUFA in adipose tissue, which has also been observed in human subjects[19], adipose tissue provides high storage capacity for these fatty acids. Thus, in lean adult human subjects adipose tissue accounts for 15–25% body weight (this percentage can increase to 50% in morbidly-obese patients), while approximately 70% of the adipose tissue mass comprises lipids[42]. Accordingly, adipose tissue is known to serve as a buffer for LC PUFA in nursing mothers, thus preventing large fluctuations of LC PUFA concentration in breast milk[43].

Extrapolation of the results relating to the dose dependence of various effects of *n*-3 LC PUFA from mice to human subjects is problematic for several reasons including, for example, a large difference in specific metabolic rate between the two species. In this context the results describing saturability of various plasma and tissue compartments with DHA may provide a useful lead, since a saturation of the plasma (phospholipid) pool by DHA has also been observed in human subjects[19]. Thus, it could be inferred that *n*-3 LC PUFA effects observed in mice fed the cHF diet at the 15% (w/w) level of substitution (35%

(w/w) fat; with 15% of its lipids replaced by the *n*-3 LC PUFA concentrate, corresponding to approximately 9 g DHA + EPA/100 g dietary lipids) are relevant for human subjects treated with about 2 g DHA (in a mixture with EPA)/d, since under these conditions the plasma lipid pool is close to saturation with DHA in both mice and human subjects[19] (see Fig. 1(A)). A decrease in the DHA:EPA[19] or in the fat content of the diet should result in the lower DHA intake needed for saturation of the plasma pool.

Prevention of body fat accumulation by *n*-3 long-chain PUFA

In accordance with other studies (for review, see Ruzickova *et al.*[11]), the experiments on C57BL/6 mice have also demonstrated that substitution of 15% (w/w) lipids in cHF diets by the *n*-3 LC PUFA concentrate EPAX 1050 TG prevents fat accumulation with a preferential reduction in abdominal fat depots[11–13]. Using semi-synthetic high-fat diets a stronger anti-obesity effect has been observed with increasing DHA:EPA in the diets[11]. The reduction in adipose tissue growth results in part from the inhibition of fat cell proliferation[11]. *In vitro*, DHA inhibits adipocyte differentiation and induces apoptosis in post-confluent preadipocytes[44]. DHA also induces apoptosis in several models of cancer[45]. The mechanism of the anti-proliferative effect of *n*-3 LC PUFA on adipose tissue is not completely understood and may reflect modulation of *in situ* eicosanoid production[46–48]. The anti-proliferative effect of *n*-3 LC PUFA may be involved in the reduced adiposity of pups born to rat or mouse dams fed diets supplemented with *n*-3 LC PUFA[49] or ALA[22] during gestation and suckling, and even in the anti-obesity[50] and anti-diabetic effects[51] of breast-feeding. Moreover, all these studies indicate that reduction in both hyperplasia of adipose tissue cells and hypertrophy of adipocytes (also, see later) contribute to the reduced accumulation of body fat as a result of *n*-3 LC PUFA intake.

Induction of a metabolic switch in adipose tissue and small intestine by *n*-3 long-chain PUFA

It has been found previously that feeding C57BL/6 mice a cHF diet supplemented with *n*-3 LC PUFA (i.e. 15% (w/w) lipids in cHF diets substituted by *n*-3 LC PUFA concentrate EPAX 1050 TG) induces mitochondrial biogenesis in white fat, with a stronger effect in epididymal fat in the abdomen than in subcutaneous adipose tissue[12]. The effect in abdominal fat is associated with a 3-fold increase in the expression of genes for regulatory factors for mitochondrial biogenesis and oxidative metabolism, PPARγ co-activator 1α and nuclear respiratory factor-1 respectively. A marked down-regulation of the stearoyl-CoA desaturase gene, *Scd-1*, is observed in white fat[12], consistent with the induction of lipid oxidation by *n*-3 LC PUFA in the tissue[12] and the role of *Scd-1*[52] in the control of lipid oxidation. Expression of PPARγ co-activator 1α and nuclear respiratory factor-1 genes is also stimulated by *n*-3 LC PUFA in 3T3-L1 adipocytes[12].

Similar to the effect in adipose tissue, mitochondrial biogenesis is also induced by *n*-3 LC PUFA in the small intestine[34] but not in the liver[12] or in the skeletal muscle (P Flachs and J Kopecky, unpublished results). Also, in the adipose tissue and in the intestine, as in the liver (see earlier), *n*-3 LC PUFA increase fatty acid oxidation, while *in situ* lipogenesis is suppressed[34]. Thus, in the liver, adipose tissue and intestine dietary intake of *n*-3 LC PUFA induces a switch toward lipid catabolism and suppressed lipogenesis. Moreover, *n*-3 LC PUFA could depress basal lipolysis in adipose tissue of obese and insulin-resistant rats fed a sucrose-rich diet[53].

The effect of *n*-3 LC PUFA on lipolysis may reflect restoration of the anti-lipolytic effect of insulin, while the induction of the metabolic switch in adipocytes could depend on transcriptional control mediated by PPARα and PPARγ[27–29]. It has been hypothesized previously[23] that the AMPK regulatory axis[38] could also be involved in the induction of the metabolic switch, including up-regulation of mitochondrial biogenesis and suppression of basal lipolysis in adipocytes. As indicated in a recent review[23], induction of AMPK activity in adipose tissue[54] is the key mechanism that induces (1) the metabolic switch toward lipid catabolism and suppressed lipogenesis in adipocytes and (2) the lean phenotype of mice with respiratory uncoupling induced by the aP2-*Ucp1* transgene in adipocytes; AMPK in adipose tissue is also activated by phosphorylation in response to the anti-diabetic drugs the thiazolidinediones, the adipokines leptin and adiponectin, starvation and physical activity, i.e. under conditions promoting the metabolic switch in white fat. Phosphorylation of AMPK is required for the full activation of the enzyme and the phosphorylated:unphosphorylated form reflects the actual enzymic activity. To evaluate possible activation of the AMPK regulatory cascade[38] in white fat by *n*-3 LC PUFA the content of α1 AMPK and phosphorylated AMPK, as well as total acetyl-CoA carboxylase (ACC) and its phosphorylated form was evaluated by Western blotting in epididymal fat of mice fed cHF substituted with *n*-3 LC PUFA at the 15% or 44% (w/w) level for 5 weeks (Fig. 2(A and B)). ACC is the target for AMPK and its activity is inhibited by AMPK-mediated phosphorylation. Both α1 AMPK and phosphorylated AMPK contents tend to increase in response to the 15% (w/w) level of substitution (not shown) and their contents increase significantly in mice fed diet at the 44% (w/w) level of substitution, while the phosphorylated AMPK: total α1 AMPK remains unchanged (Fig. 2(A)). The contents of both total ACC and phosphorylated ACC, as well as the phosphorylated ACC: total ACC increase in response to the cHF diet at the 44% (w/w) level of substitution (Fig. (2B)). The status of AMPK phosphorylation is known to change in response to various immediate stimuli, while corresponding changes in ACC phosphorylation are more stable and could serve as a better marker of the metabolic switch. Activation of AMPK has also recently been observed in genetically-obese *ob/ob* mice (B6.V-Lep^ob^/J) fed a low-fat (7% (w/w) fat) diet supplemented with *n*-3 LC PUFA[24]. Thus, although no difference in phosphorylated AMPK: AMPK was detected in the experiment, the results together suggest activation of the AMPK intracellular regulatory
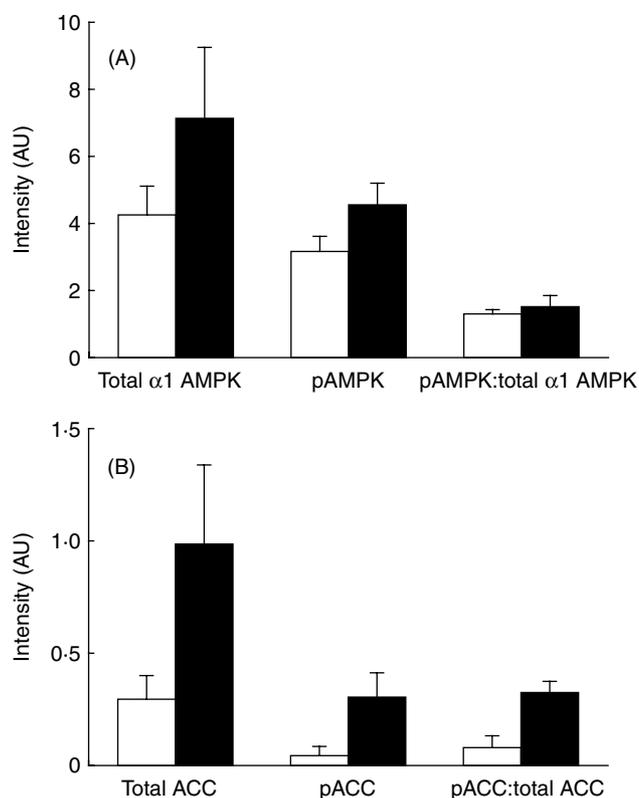

Fig. 2. AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC) phosphorylation. At 3 months of age mice were randomly assigned to a maize-based high-fat (cHF) diet or the cHF diet with 44% (w/w; D44) of its lipid replaced by *n*-3 long-chain PUFA concentrate EPAX 1050 TG (EPAX AS, Aalesund, Norway). After 5 weeks of feeding the mice were killed and epididymal adipose tissue collected for the analysis of AMPK and ACC phosphorylation[54,76]. (A), Total α1 AMPK-immunoreactive protein, phosphorylated form of AMPK (pAMPK) and pAMPK:total AMPK, measured as a signal intensity using Western blotting. (B), Total ACC-immunoreactive protein, phosphorylated form of ACC (pACC) and pACC: total ACC, measured as a signal intensity using Western blotting. (□), Control cHF diet; (■), experimental D44 diet. Values are mean with their standard errors represented by vertical bars for five to eight mice. AU, arbitrary units.

pathway by *n*-3 LC PUFA. As shown in the case of the activation of AMPK in adipocytes under other conditions (see earlier), *n*-3 LC PUFA can also promote the conversion of white adipocytes into 'fat burning cells' by this mechanism[55,56]. Activation of AMPK could increase the mitochondrial content of adipocytes, which is consistent with the suggestion 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[57].

The AMPK-dependent induction of the metabolic switch in white fat should contribute to a decrease in the size of mature adipocytes, as observed in mice treated with *n*-3 LC PUFA or thiazolidinediones[11,58,59] and even in patients with diabetes whose diet is supplemented with *n*-3 LC PUFA[60]. Compared with large adipocytes, small cells are more insulin sensitive and less lipolytic, release lower
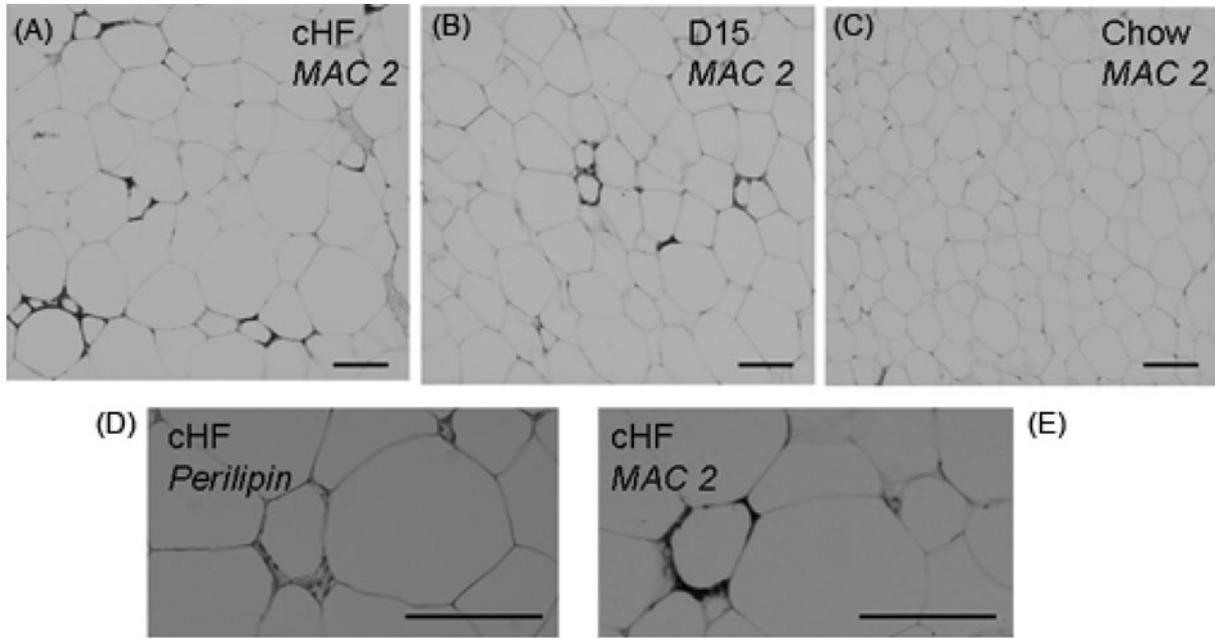
Fig. 3. Immunodetection of macrophages in epididymal adipose tissue. At 3 months of age mice were placed on a maize-based high-fat diet (cHF) or the cHF diet with 15 % (w/w; D15) of its lipid replaced by *n*-3 long-chain PUFA concentrate EPAX 1050 TG (EPAX AS, Aalesund, Norway). Some mice were maintained on a chow diet. After 20 weeks of treatment, mice were killed and adipose tissue samples processed for immunohistological analysis[67]. (A, B, C), MAC-2 (β-galactoside-binding lectin expressed on activated macrophages)-immunoreactive macrophages were detected in 5 μm thick sections (darkly-stained cells). Note that almost all macrophages are localized within crown-like structures surrounding individual adipocytes. (A), cHF diet; (B), D15 diet; (C), chow diet; (D, E), detailed view of adipose tissue from the cHF-fed mice with visualized perilipin and MAC-2 respectively. —, 200 μm.

levels of inflammatory cytokines (for review, see Yang & Smith[61]) and secrete higher levels of adiponectin[62]. The small cells could also serve as a 'buffer' for lipids and protect tissues against the lipotoxicity[63,64]. Thus, *n*-3 LC PUFA would enhance insulin action in adipose tissue by counteracting adipose tissue hypertrophy. Despite a minimal contribution of adipose tissue to the whole-body glucose uptake, impairment of glucose transport in adipocytes results in insulin resistance in the skeletal muscle and liver[65]; the insulin-sensitizing effect of *n*-3 LC PUFA in adipocytes may be crucial for the beneficial effect of these lipids on whole-body glycaemic control. Importantly, in addition, induction of the metabolic switch in the small intestine by *n*-3 LC PUFA could be mediated by AMPK and limit accretion of body fat[34,37].

Amelioration of low-grade inflammation of adipose tissue and induction of adiponectin by *n*-3 long-chain PUFA

In accordance with the general anti-inflammatory action of *n*-3 LC PUFA[6] (possibly mediated by NF-κB[7,34]) low-grade inflammation of adipose tissue, which is associated with obesity, is also reduced by *n*-3 LC PUFA supplementation in obese diabetic *db/db* mice[59], as well as in the experiments on C57BL/6 mice fed the cHF diet at the 15 % (w/w) level of substitution[13]. The inflammation is suppressed even more potently by a DHA derivative (α-ethyl DHA ethyl ester) replacing only 1·5 % (w/w)

lipids in the cHF diet[66]. As shown in Fig. 3, the cHF diet-induced adipose tissue hypertrophy is associated with infiltration of adipose tissue by macrophages immunoreactive for MAC-2 (β-galactoside-binding lectin expressed on activated macrophages), which aggregate in crown-like structures surrounding adipocytes[67]. Numerous crown-like structures are detected in cHF diet-fed mice (Fig. 3(A and E)) but not in chow diet-fed (Fig. 3(C)) mice. In accordance with the previous study[67], adipocytes surrounded by crown-like structures are not viable, based on the absence of perilipin staining. Viable adipocytes, not surrounded by macrophages, are positive for perilipin (Fig. 3(D)). *n*-3 LC PUFA prevent, in part, the macrophage infiltration (Fig. 3(B)). It should be investigated whether *n*-3 LC PUFA not only decrease the total number of macrophages in adipose tissue but could also change their polarity, while suppressing specifically M1 pro-inflammatory macrophages and activating M2 macrophages secreting anti-inflammatory cytokines[68]. Such an effect has recently been demonstrated for thiazolidinediones, affecting macrophages in adipose tissue[69] and peripheral blood[70].

The suppression of the low-grade inflammation of adipose tissue by *n*-3 LC PUFA in mice is associated with induction of adiponectin[32,71,72], the major adipokine exerting an insulin-sensitizing effect, possibly as a result of activation of AMPK in various tissues[73,74]. The induction of adiponectin is stronger in the epididymal fat in the abdomen than in the subcutaneous fat[32,72] and it is probably mediated by PPARγ[32] in fully-differentiated[72] fat

cells. A recent clinical study has demonstrated the induction of plasma adiponectin in response to a daily intake of 1·3 g EPA and 2·9 g DHA (administered as EPAX 2050 TG; EPAX AS) in overweight patients who were simultaneously undertaking a weight-loss programme[75]. The induction of adiponectin could contribute to the beneficial effect of *n*-3 LC PUFA on systemic insulin sensitivity.

It has recently been investigated whether combined treatment with DHA + EPA and the thiazolidinedione rosiglitazone would provide additive beneficial effects on various features of MS in the cHF diet-fed mice[13]. DHA + EPA administered at the 15 % (w/w) level of substitution and a low dose of rosiglitazone exert additive effects in the prevention of obesity, adipocyte hypertrophy, low-grade adipose tissue inflammation, induction of adiponectin, dyslipidaemia and insulin resistance. The combined treatment also reverses dietary obesity, dyslipidaemia and impaired glucose tolerance in the mice. These results suggest that DHA + EPA and thiazolidinediones could be used as complementary therapies to counteract various pathologies associated with MS and that adipose tissue represents an important target in this strategy.

Conclusion

Adipose tissue metabolism, inflammatory status and secretion of adipokines play an important role in the development of the pathological conditions associated with MS. Despite a relatively small increase in *n*-3 LC PUFA concentrations in adipose tissue lipids in response to dietary intake of these fatty acids, adipose tissue possesses a substantial capacity for *n*-3 LC PUFA storage, and it is specifically linked to the beneficial effects of *n*-3 LC PUFA on health. Surprisingly strong suppression of adipose tissue hyperplasia and hypertrophy by *n*-3 LC PUFA supplementation, reflecting induction of lipid catabolism and suppression of lipogenesis in adipocytes, as well as amelioration of adipose tissue inflammation and increased secretion of adiponectin, help to explain the beneficial effects of *n*-3 LC PUFA in the prevention and treatment of various components of MS. Modulation of adipose tissue metabolism, cellular composition and secretion of adipokines by *n*-3 LC PUFA has a prominent role in the multiple mechanisms of action of these lipids and should be explored further in combination therapies for various pathologies in human subjects.

Acknowledgements

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