Conference on ‘Over- and undernutrition: challenges and approaches’

Postgraduate Symposium

The role of inflammation and macrophage accumulation in the development of obesity-induced type 2 diabetes mellitus and the possible therapeutic effects of long-chain n-3 PUFA

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The WHO estimate that >1 × 10\textsuperscript{6} deaths in Europe annually can be attributed to diseases related to excess body weight, and with the rising global obesity levels this death rate is set to drastically increase. Obesity plays a central role in the metabolic syndrome, a state of insulin resistance that predisposes patients to the development of CVD and type 2 diabetes mellitus. Obesity is associated with low-grade chronic inflammation characterised by inflamed adipose tissue with increased macrophage infiltration. This inflammation is now widely believed to be the key link between obesity and development of insulin resistance. In recent years it has been established that activation of pro-inflammatory pathways can cross talk with insulin signalling pathways via a number of mechanisms including (a) down-regulation of insulin signalling pathway proteins (e.g. GLUT4 and insulin receptor substrate (IRS)-1), (b) serine phosphorylation of IRS-1 blocking its tyrosine phosphorylation in response to insulin and (c) induction of cytokine signalling molecules that sterically hinder insulin signalling by blocking coupling of the insulin receptor to IRS-1. Long-chain (LC) n-3 PUFA regulate gene expression (a) through transcription factors such as PPAR and NF-kB and (b) via eicosanoid production, reducing pro-inflammatory cytokine production from many different cells including the macrophage. LC n-3 PUFA may therefore offer a useful anti-inflammatory strategy to decrease obesity-induced insulin resistance, which will be examined in the present review.

Obesity-induced insulin resistance: Inflammation: Adipose tissue macrophages: Long-chain n-3 PUFA

Obesity can simply be defined as a condition of excessive fat accumulation in adipose tissue, which causes or exacerbates many health problems, both independently and in association with other diseases\textsuperscript{(1)}. Half all adults and one in five children in Europe are now overweight, one-third of whom are obese\textsuperscript{(2)}. In Europe >1 × 10\textsuperscript{6} deaths annually are attributable to diseases related to excess body weight\textsuperscript{(2)}. Obesity plays a central role in insulin-resistant states such as type 2 diabetes mellitus (T2DM). In the SEARCH for Diabetes in Youth Study of 3953 individuals with T2DM 10.4\% were shown to be overweight and 79.4\% obese, illustrating that obesity is a major contributing factor in T2DM\textsuperscript{(3)}. In insulin-resistant states signal transduction via the insulin receptor is impaired, with decreased activation of downstream targets such as insulin receptor substrate (IRS)-1 and protein kinase B, which are involved in stimulating translocation of GLUT4 to the cell surface\textsuperscript{(4)}.

Recent studies have shown that obesity gives rise to a state of chronic low-grade inflammation characterised by inflamed adipose tissue with increased infiltration of.
macrophages that produce pro-inflammatory cytokines\(^{(5,6)}\). These cytokines, such as TNF\(\alpha\), directly reduce insulin sensitivity through the insulin-signalling pathway\(^{(7)}\). Macrophage-secreted factors block insulin action in adipocytes via down-regulation of GLUT4 and IRS-1\(^{(8)}\). It is therefore proposed that the adipose tissue macrophages (ATM) may directly contribute to insulin resistance observed in obesity.

Long-chain (LC) \(n-3\) PUFA can exert anti-inflammatory effects by reducing pro-inflammatory cytokine expression in many chronic inflammatory conditions. Thus, LC \(n-3\) PUFA may offer a useful anti-inflammatory strategy to decrease obesity-related disease\(^{(9)}\). The anti-inflammatory actions of LC \(n-3\) PUFA may be (a) direct, such as their action on transcription factors influencing gene expression, or (b) mediated through eicosanoid production. The present review will begin by briefly examining the central role of obesity in T2DM and will then describe the crucial function of insulin signalling in both cell biology and T2DM pathology. The discussion will then focus on the role of the macrophage within obesity, exploring the molecular mechanisms that mediate the pro-inflammatory interaction between macrophages and adipocytes in obesity. Finally, the review will discuss whether LC \(n-3\) PUFA can attenuate the pro-inflammatory and insulin-resistant phenotype observed in obesity. However, it is important to mention that much of the evidence examining the protective effects of LC \(n-3\) PUFA in a T2DM environment remains unclear and requires further investigation.

### Obesity and type 2 diabetes

Obesity plays a central role in the metabolic syndrome, which includes hyperinsulininaemia, hypertension and hyperlipidaemia with an increased risk of CVD and T2DM\(^{(10)}\). The adverse metabolic changes associated with obesity are mostly related to a reduction in sensitivity of the body’s tissues to insulin, the state termed insulin resistance. The risk of T2DM increases with greater BMI. The Nurses’ Health Study has found that after adjustment for age BMI is the dominant predictor of risk for T2DM\(^{(11)}\). The risk of diabetes increases 5-fold for women with a BMI of 25 kg/m\(^2\), 28-fold for those with a BMI of 30 kg/m\(^2\) and 93-fold for those women with BMI of \(\geq 35\) kg/m\(^2\) when compared with women with a BMI of \(<21\) kg/m\(^2\). A strong positive association between overall obesity as measured by BMI and risk of T2DM has also been found in men\(^{(12)}\). Men with a BMI of \(\geq 35\) kg/m\(^2\) have a 42-fold increased risk of T2DM compared with men with a BMI of \(<23\) kg/m\(^2\). The association between obesity and the metabolic syndrome and CVD risk is not only related to BMI but seems to be critically dependent on body fat distribution. Individuals with greater extents of central adiposity or visceral adipose tissue develop metabolic syndrome more frequently than individuals with a peripheral body fat distribution\(^{(13)}\).

T2DM is characterised by peripheral insulin resistance, increased hepatic glucose production and impaired insulin secretion\(^{(14)}\). T2DM, once seen as a relatively mild ailment associated with ageing and the elderly, is now considered to be a chronic and debilitating disease. T2DM is ranked among the leading causes of blindness, renal failure and lower limb amputation, and through its effects on CVD it is also now considered to be one of the leading causes of death\(^{(15)}\). The life expectancy of individuals with T2DM can be shortened by \(\leq 15\) years, with \(\leq 75\%\) dying of CVD\(^{(16)}\). Insulin, a hormone secreted by the pancreas, affects a wide range of biological processes including glucose transport, glucose and lipid metabolism, cell growth, protein synthesis and gene expression in many different cell types and multiple organs including the liver, muscle and adipose tissue\(^{(17)}\).

### Insulin signalling

Impaired signal transduction via the insulin receptor in insulin-resistant states\(^{(4)}\) results in a decrease in insulin-stimulated glucose transport and metabolism in adipocytes and skeletal muscle, with impaired suppression of hepatic glucose output\(^{(18)}\). However, insulin has many more effects at both a cell signalling and gene expression level, including its effects on carbohydrate, lipid and protein metabolism\(^{(17)}\). Thus, a decrease in insulin sensitivity undoubtedly has many serious and widespread consequences within the body. A brief outline of the insulin signalling pathway is shown in Fig. 1.

Insulin acts by binding to its cell surface receptor; the principal IRS proteins, IRS-1 and IRS-2, are phosphorylated on multiple tyrosine residues by the active receptors for insulin, insulin-like growth factor-1 and various other cytokines\(^{(19)}\). Tyrosine phosphorylation of IRS-1 and IRS-2 promotes their binding to the Shc homology-2 domains in various downstream signalling proteins including phosphatidylinositol 3-kinase and growth factor receptor-bound protein-2\(^{(19)}\). During association with IRS proteins phosphatidylinositol 3-kinase is activated and its phospholipid products promote the recruitment of various serine kinases such as protein kinases B and C to the plasma membrane, where they are activated by phosphorylation\(^{(20)}\). Protein kinases B and C phosphorylate multiple downstream effectors that promote diverse biological responses including: GLUT4 translocation at the plasma membrane\(^{(21)}\); glycogen synthesis via protein kinase B-mediated inhibitory phosphorylation of glycogen synthase kinase-3, which negatively regulates glycogen synthase\(^{(22)}\); lipogenesis via up-regulation of the expression of the fatty acid synthase gene\(^{(23)}\); more general control of gene expression patterns\(^{(24)}\). GLUT4 is the predominant GLUT isoform expressed in mature muscle and fat tissues and is primarily responsible for enhanced glucose uptake in response to insulin\(^{(25)}\). However, serine phosphorylation of IRS-1 via serine/threonine kinases results in an impaired ability of insulin to phosphorylate the tyrosine residues of IRS-1. The phosphorylation state of IRS-1 Ser307 (in rodents) or Ser312 (in human subjects) might predict the ability of IRS-1 to mediate the insulin response\(^{(26)}\). Interestingly, activation of c-Jun N-terminal kinase by pro-inflammatory cytokines inhibits insulin signalling, at least in part, by stimulating phosphorylation of IRS-1 at Ser307 (in mice) and Ser312 (in human subjects)\(^{(26,27)}\). The mitogen-activated protein kinase pathways are also activated by...
insulin, via both Shc association with the insulin receptor and growth factor receptor-bound protein-2 association with both the insulin receptor and IRS molecules. The extracellular signal-regulated kinase 1/2 does not seem to play a major role in mediating insulin’s metabolic responses; however, increased basal mitogen-activated protein kinase activity appears to contribute to the development of insulin resistance. Conversely, p38 mitogen-activated protein kinase activity has been proposed as a positive regulator of insulin action because of its capability to increase the uptake of glucose via GLUT4\(^{28}\).

Insulin action in adipocytes also involves changes in gene transcription. The transcription factor adipocyte determination and differentiation factor-1/sterol regulatory element binding protein (SREBP) and PPAR. Tyrosine phosphorylation of insulin receptor substrate (IRS)-1 and SHC on the insulin receptor activate phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) signalling. In combination these pathways regulate glucose, lipid and protein metabolism. GRB2, growth factor receptor-bound protein-2; PKB, protein kinase B; GSK3, glycogen synthase kinase-3; JNK, c-Jun N-terminal kinase; \(\rightarrow\), Activation; \(\leftarrow\), inhibition; \(\downarrow\), uptake.

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**Fig. 1.** Activation of the insulin receptor evokes increased transcription of sterol regulatory element binding protein (SREBP) and PPAR. Tyrosine phosphorylation of insulin receptor substrate (IRS)-1 and SHC on the insulin receptor activate phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) signalling. In combination these pathways regulate glucose, lipid and protein metabolism. GRB2, growth factor receptor-bound protein-2; PKB, protein kinase B; GSK3, glycogen synthase kinase-3; JNK, c-Jun N-terminal kinase; \(\rightarrow\), Activation; \(\leftarrow\), inhibition; \(\downarrow\), uptake.

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Obesity: a chronic pro-inflammatory state

Adipose tissue produces a number of cytokines and bioactive molecules, which together are termed adipokines\(^{34}\). Some adipokines act in an autocrine or paracrine manner, while others are released into the systemic circulation and act as signalling molecules in other tissues. Compared with the adipose tissue of lean individuals, that of obese subjects expresses increased amounts of pro-inflammatory proteins such as TNF\(\alpha\), IL-6, inducible NO synthase, transforming growth factor \(\beta1\), C-reactive protein, soluble intercellular adhesion molecule, monocyte chemotactic protein (MCP)-1, plasminogen activator inhibitor type 1 tissue factor and factor VII\(^{35–41}\). Adiposity is negatively correlated with production of adiponectin, an insulin-sensitising hormone that decreases hepatic gluconeogenesis and increases lipid oxidation in muscle\(^{42,43}\). Recent data suggest that in adipose tissue pro-inflammatory molecules, including IL-1\(\beta\), PGE\(_2\), TNF\(\alpha\) and IL-6, are produced
by stromal vascular cells, which include pre-adipocytes, vascular cells (such as endothelial cells) and immune cells. A major conceptual advance in the field of obesity-induced inflammation and insulin resistance was made by the discovery that obesity gives rise to a state of chronic low-grade systemic inflammation with evidence of increased infiltration of macrophages into the adipose tissue. Microarray analyses comparing adipose tissue RNA profiles of various mouse models of obesity have identified a subset of genes consistently expressed in obese mice, with further analyses showing that this gene set, not typically expressed in adipocytes, is macrophage derived. Using immunohistochemical analysis of perigonadal, perirenal, mesenteric and subcutaneous adipose tissue it has been shown that the percentage of cells expressing the macrophage marker F4/80 (F4/80$^{+}$) is substantial and positively correlated with both adipocyte size and body mass. Furthermore, the F4/80$^{+}$ cells have been shown to be colony-stimulating factor-1-dependent bone marrow-derived ATM. Similar findings have been reported by other investigators who have also shown that thiazolidinedione treatment represses the expression of macrophage-specific genes, providing an additional mechanism by which thiazolidinedione treatment improves insulin sensitivity. The ATM have been shown to produce many of the pro-inflammatory molecules released by adipose tissue, including TNFα and a substantial portion of NO synthase 2 and IL-6 gene expression. Pro-inflammatory cytokines such as TNFα, IL-1β and IL-6 have been implicated in the development of insulin resistance and the pathophysiology of T2DM and obesity. In both human subjects and rodents ATM accumulate in adipose tissue with increasing body weight and their quantity correlates with measures of insulin resistance. In obese subjects ATM content is higher in visceral adipose tissue than in subcutaneous adipose tissue, consistent with the hypothesis that visceral fat plays a more prominent role in insulin resistance.

### Adipose tissue macrophages in obesity

Both adipocyte hyperplasia and hypertrophy can contribute to adipose tissue expansion; however, in adults hypertrophy appears to predominate. Some of the consequences of hypertrophy include vascularisation, hypoxia and adipocyte cell death. Furthermore, other immune cells, such as neutrophils and T-cells, may enter the adipose tissue first and contribute to macrophage recruitment. These effects all combine to cause macrophage recruitment into adipose tissue, as shown in Fig. 2. Obese mouse models such as diet-induced obese mice and leptin-deficient ob/ob mice have been used to...
demonstrate that hypoxia occurs in obese adipose tissue\(^{(47,48)}\). Decreased vascular density that has been observed in obese mice\(^{(49)}\) may contribute to hypoxia. Importantly, it has been suggested that the ATM may act to stimulate angiogenesis in the adipose tissue\(^{(50)}\), which could be a rationale for why the macrophage infiltrate adipose tissue. Furthermore, mRNA and protein levels of hypoxia-inducible factor-1\(\alpha\) are elevated in adipose tissue of obese mice and obese human subjects, as are mRNA and protein levels for other hypoxia-inducible genes\(^{(47,48,51,52)}\). It has been demonstrated in vitro that hypoxia may contribute to adipose tissue inflammation by showing that exposure of primary adipocytes and macrophages to hypoxia increases their expression of multiple inflammatory genes\(^{(48)}\).

It has been demonstrated that >90% of all macrophages in white adipose tissue are localised to dead adipocytes, where they fuse to form syncytia that sequester and scavenge the residual ‘free’ adipocyte lipid droplets and ultimately form multinucleated giant cells, a hallmark of chronic inflammation\(^{(53)}\). Adipocyte death increases 30-fold in obese leptin-deficient \(ob/ob\) mice and obese human subjects exhibit ultrastructural features of necrosis\(^{(53)}\). Necrotic-like adipocyte cell death is a pathological hallmark of obesity and suggests that scavenging of adipocyte debris is an important function of the ATM in obese individuals\(^{(53)}\).

Chemokines are small chemotactic cytokines that are well established to play a role in macrophage mobilisation out of bone marrow and into many different tissues during the inflammatory process\(^{(46)}\). Although they can be secreted by adipocytes, studies in which adipocytes are separated from the stromal vascular cell fraction have demonstrated that the majority of chemokine secretion in adipose tissue is from the stromal vascular cell fraction\(^{(54)}\). Thus, expression of chemokines from ATM may contribute to propagation of macrophage accumulation in the adipose tissue\(^{(56)}\). Circulating concentrations of the chemokine MCP-1, also known as CCR2, are increased in obese subjects\(^{(54)}\) and are elevated in patients with T2DM compared with patients who do not have T2DM\(^{(55)}\). It has been demonstrated that in obese mice matched for adiposity Ccr2 deficiency reduces ATM content and the inflammatory profile of adipose tissue and there is increased adiponectin expression, ameliorated hepatic steatosis and insulin sensitivity\(^{(56)}\).

Pro- and anti-inflammatory adipose tissue macrophages

The capability of macrophages to secrete both pro- and anti-inflammatory cytokines contributes to their dual role, and ingestion of apoptotic cells has been shown to programme macrophages to become anti-inflammatory\(^{(57)}\). Different stimuli activate macrophages to express distinct patterns of chemokines, surface markers and metabolic enzymes that ultimately generate the diversity of macrophage function seen in pro-inflammatory and anti-inflammatory settings\(^{(58)}\). Macrophage activation has been operationally defined across two separate polarisation states, M1 and M2\(^{(59,60)}\). M1 (‘classically-activated’) macrophages are induced by pro-inflammatory mediators such as lipopolysaccharides and IFN-\(\gamma\), have enhanced pro-inflammatory cytokine production (TNF\(\alpha\), IL-6 and IL-12) and generate reactive oxygen species such as NO via activation of inducible NO synthase. M2 (‘alternatively-activated’) macrophages are generated in vitro by exposure to IL-4 and IL-13, have low pro-inflammatory cytokine expression and generate high levels of anti-inflammatory cytokines IL-10 and IL-1 receptor\(^{(59)}\). F4/80+ CD11c+ populations of ATM have been identified\(^{(58)}\). These macrophages are thought to be M1 macrophages and have been found in the adipose tissue of obese mice and not in lean mice\(^{(58)}\). ATM from lean mice express many genes characteristic of M2 macrophages, including IL-10. Interestingly, diet-induced obesity decreases expression of these characteristic M2 genes in ATM and increases expression of genes such as those encoding TNF\(\alpha\) and inducible NO synthase, which are characteristic of M1 macrophages. It has been found that ATM from obese Ccr2\(^{-/-}\)-knock-out mice, which have reduced macrophage infiltration into the adipose tissue, express M2 markers at levels similar to those from lean mice\(^{(58)}\), suggesting that MCP-1 maybe an important factor in regulating macrophage activation. Interestingly, a macrophage-specific deficiency of PPAR\(\gamma\) results in an inability to develop the alternatively-activated M2 phenotype\(^{(61)}\). Macrophage-specific PPAR\(\gamma\)-knock-out mice show a predisposition to diet-induced weight gain, glucose intolerance and insulin resistance. Despite the increased adipose tissue mass macrophage-specific PPAR\(\gamma\)-knock-out mice have reduced total ATM, which appears to be the result of a reduction in M2 macrophages\(^{(61)}\). This finding suggests that M2 macrophages provide protection against diet-induced insulin resistance and that PPAR\(\gamma\) is fundamental to macrophages becoming the M2 phenotype. Human macrophage populations cannot always be classified simply as M1 or M2. However, it has been demonstrated that human ATM have both M1 and M2 characteristics, as evidenced by their secretion of both pro- and anti-inflammatory cytokines\(^{(23,62)}\).

Interactions between macrophages and adipocytes

An examination has been made of how macrophages and adipocytes interact in vitro and whether macrophages can modify insulin responsiveness and glucose metabolism in adipocytes\(^{(8)}\). Macrophage-secreted factors reduce insulin-stimulated glucose uptake in adipocytes via down-regulation of GLUT4 and IRS-1. Furthermore, insulin-stimulated plasma membrane translocation of GLUT4 is attenuated by macrophage-secreted factors. Treatment of macrophage-conditioned medium with TNF\(\alpha\)-blocking antibodies partially reverses this insulin-resistant state\(^{(8)}\). TNF\(\alpha\) induces the expression of a variety of inflammatory cytokines in adipocytes, including IL-6, plasminogen-activator inhibitor-1, MCP-1 and TNF\(\alpha\) itself\(^{(60)}\). Thus, the induction of insulin inhibitory effects of TNF\(\alpha\) may not be direct. However, it has been shown that pro-inflammatory cytokines such as IL-6, macrophage-inflammatory...
protein-2 and MCP-1 are induced in macrophages within a co-culture of adipocytes and macrophages(66).

TNFα reduces insulin-stimulated receptor tyrosine kinase activity at low concentrations and can also decrease the expression of the insulin receptor, IRS-1 and GLUT-4 at higher concentrations as well as increase the phosphorylation of serine 307 of IRS-1, thus impairing its ability to bind to the insulin receptor and initiate downstream signalling(64). IL-6, like TNFα, exerts long-term inhibitory effects on the gene transcription of IRS-1, GLUT-4 and PPARγ in adipocytes(64). This effect of IL-6 is accompanied by a marked reduction in IRS-1 protein expression and reduction in insulin-stimulated IRS-1 tyrosine phosphorylation coincident with impaired insulin-stimulated glucose transport(65). TNFα increases IL-6 mRNA and protein secretion in adipocytes(65). TNFα and IL-6 also enhance the expression of suppressor of cytokine signalling 1 and 3 molecules that can attenuate insulin signalling by sterically hindering coupling of insulin receptor with IRS-1(66). Suppressor of cytokine signalling proteins can also bind directly to IRS-1, facilitating its ubiquitination and subsequent degradation by the proteasome(67). Interestingly, the addition of the chemokine MCP-1 to differentiated adipocytes in vitro decreases insulin-stimulated glucose uptake and the expression of several adipogenic genes, including GLUT4 and PPARγ, which may suggest that elevated MCP-1 may induce adipocyte dedifferentiation that would contribute to a reduction in insulin sensitivity(49).

Several kinases including c-Jun N-terminal kinase(26), mammalian target of rapamycin and extracellular signal-regulated kinases(68) have been implicated in the serine phosphorylation or deactivation of IRS-1(69). Many of these mitogen-activated protein kinases including c-Jun N-terminal kinase, extracellular signal-regulated kinases and p38 are activated by pro-inflammatory cytokines.

NF-κB is a transcription factor that plays a major role in inducing a range of inflammatory genes including cyclooxygenase-2, intercellular adhesion molecule-1, vascular cell adhesion molecule, E-selectin, TNFα, IL-1β, IL-6, inducible NO synthase, acute-phase proteins and matrix metalloproteinases in response to inflammatory stimuli(70,71). NF-κB is an essential factor in acute as well as chronic inflammation and is also activated by pro-inflammatory cytokines. Macrophage-derived cytokines may therefore induce NF-κB activation within the adipocytes of the adipose tissue, exacerbating the pro-inflammatory environment. Mice with a myeloid-specific knock-out of inhibitor of NF-κB kinase β (an activator of NF-κB) are protected from obesity-induced diabetes, clearly demonstrating the importance of inflammation in modulating insulin sensitivity specifically through the NF-κB pathway(72).

The anti-inflammatory effects of long-chain n-3 PUFA

Many human LC n-3 PUFA intervention studies have shown anti-inflammatory effects in patients with chronic inflammatory conditions such as rheumatoid arthritis(73), asthma(74), Crohn’s disease(75) and psoriasis(76), and LC n-3 PUFA have been shown to alleviate symptoms of each disease. The evidence for the beneficial effects of LC n-3 PUFA within clinical trials is often conflicting, which may be a result of factors such as the medical condition under analysis, the size of the study, the specific cytokines examined within the study, the study end points and the LC n-3 PUFA dose (for a review of many of these concepts, see Sijben & Calder(77)). On the basis of estimates from studies on Paleolithic nutrition and modern-day hunter–gatherer populations it appears that humans have evolved while consuming a diet that was much lower in SFA than today’s diet(78). Furthermore, the diet contained small and approximately equal amounts of n-6 and n-3 PUFA and much lower amounts of trans-fatty acids than does today’s diet(78,79). The current Western diet is very high in n-6 fatty acids, which is thought to have detrimental health consequences.

Linoleic acid (18:2n-6) is the major n-6 PUFA and α-linolenic acid (18:3 n-3) is the major n-3 PUFA. In the body linoleic acid is metabolised to arachidonic acid (AA; 20:4n-6) and α-linolenic acid is metabolised to EPA (20:5n-3) and DHA (22:6n-3), both LC n-3 PUFA. Linoleic acid and α-linolenic acid and their LC derivatives are important components of animal and plant cell membranes. importantly, when human subjects ingest fish or fish oil, the ingested EPA and DHA partially replace the n-6 fatty acids, particularly AA, in the cell membranes. The PUFA composition of cell membranes is therefore to a great extent dependent on dietary intake.

The n-6 and n-3 fatty acids are converted into eicosanoids (Fig. 3); therefore, the composition of the cell membrane influences eicosanoid metabolism. Eicosanoids are involved in modulating the intensity and duration of inflammatory responses(80). DHA and EPA are competitive substrates for the enzymes and products of AA metabolism. The difference between LC n-3- and n-6 PUFA-derived eicosanoids is that most of the mediators formed from EPA and DHA are anti-inflammatory, whereas those formed from AA are pro-inflammatory or show other disease-propagating effects(81). For example, PGE2 produced by AA induces fever, pain, vasodilation and vascular permeability, while leukotriene B4 also produced by AA is chemotactic for leucocytes and induces the release of reactive oxygen species by neutrophils and inflammatory cytokines (TNFα, IL-1β, IL-6) by macrophages(82,83). Furthermore, the eicosanoid metabolic products from AA are formed in larger quantities than those formed from LC n-3 PUFA(84). The recognition that EPA and DHA have anti-inflammatory properties suggests that increasing their intake corrects the LC n-6 and n-3 PUFA balance and so may act to decrease the risk of inflammatory conditions and may be of benefit to patients with inflammatory diseases(77,80).

The composition of LC n-6 and n-3 PUFA also affects gene expression and intercellular cell-to-cell communication. The balance of n-3 and n-6 PUFA is important for homeostasis and normal development within cells. PUFA rapidly modulate gene expression in different systems by regulating transcription factors such as PPAR, liver X receptors and sterol regulatory element binding protein-1(85,86). These nuclear receptors play crucial roles in the regulation of fatty acid metabolism. Liver X receptors
activate expression of sterol regulatory element binding protein-1c, a dominant lipogenic gene regulator, whereas PPAR promotes fatty acid β-oxidation gene expression. PPARγ functions in lipid catabolism and homeostasis in the liver while PPARγ appears to have a primary role in adipocyte differentiation. PPARγ agonists such as thiazolidinedione increase insulin sensitivity and are useful for treating human diabetes. PUFA are potent PPAR activators leading to the increased expression of genes responsible for fatty acid oxidation such as acyl-CoA oxidase, fatty acyl-CoA synthetase and hydroxymethylglutaryl-CoA synthase. Activators of PPAR have been shown to inhibit the activation of inflammatory genes including TNFα, IL-1β, IL-6, IL-8, cyclooxygenase-2, vascular cell adhesion molecule-1, inducible NO synthase, matrix metalloproteinases and acute-phase proteins. Two mechanisms for the anti-inflammatory actions of PPAR have been proposed. The first mechanism is that PPAR might stimulate the breakdown of inflammatory eicosanoids through induction of peroxisomal β-oxidation. The second mechanism is that PPAR might interfere with or antagonise the activation of other transcription factors, including NF-κB.

LC n-3 PUFA can also down regulate the activity of NF-κB directly, which may provide an explanation of how LC n-3 PUFA reduce inflammatory cytokine production. Feeding mice fish oil results in a lower level of NF-κB in the nuclei of lipopolysaccharide-stimulated spleen lymphocytes compared with feeding maize oil. It has been shown that in cell culture pretreatment with EPA and DHA decreases lipopolysaccharide-stimulated THP-1 macrophage TNFα, IL-1β and IL-6 production compared with control cells. Furthermore, EPA and DHA down regulate lipopolysaccharide-induced NF-κB–DNA binding in THP-1 macrophages by approximately 13%. DHA decreases macrophage nuclear p65 expression and increases cytoplasmic inhibitor of NF-κB expression. This capacity for LC n-3 PUFA to reduce inflammatory cytokine production from inflammatory cells such as the macrophages may have an important potential for reducing the inflammation induced by the ATM in obesity, improving insulin resistance.

It has been demonstrated that many genes involved in inflammatory alterations are up regulated in a T2DM mouse model, the db/db mouse, which has a defective leptin receptor when fed an HFD rich in SFA and MUFA compared with a low-fat diet. Macrophage infiltration of adipose tissue is markedly enhanced by an HFD rich in SFA and MUFA. Inclusion of LC n-3 PUFA in the diet completely prevents macrophage infiltration induced by an HFD and altered inflammatory gene expression while reducing c-Jun N-terminal kinase phosphorylation in mice with diabetes despite unreduced body weight. Furthermore, both the HFD rich in SFA and MUFA and the HFD with LC n-6 PUFA down-regulate expression of adiponectin and reduce serum concentrations, in contrast to the HFD with LC n-3 PUFA. These data suggest that beneficial effects of LC n-3 PUFA on diabetes development could be mediated by their effect on macrophage infiltration of adipose tissue and subsequent inflammation. Toll-like receptor (TLR) 4 may be an important regulator of this effect, as it has been shown to be a receptor for SFA and can mediate inflammatory cytokine production in macrophages exposed to PUFA. LC n-3 PUFA protect against TLR4-induced inflammatory cytokine production associated with SFA. Female C57BL/6 mice lacking TLR4 have increased obesity but are partially protected against HFD-induced insulin resistance. However, macrophage-specific TLR4-knock-out M0TLR4−/− mice and M0TLR4+/+ mice have similar macrophage...
accumulation in white adipose tissue and insulin sensitivity when fed an HFD (104).

**Insulin-sensitising long-chain n-3 PUFA**

Epidemiological studies have reported a low prevalence of impaired glucose tolerance and T2DM in populations consuming large amounts of LC n-3 PUFA such as the Greenland Inuit and Alaskan natives (105–107). However, much of the clinical evidence for the positive effects of LC n-3 PUFA on insulin sensitivity are conflicting and as discussed previously this disparity could be related to variable factors within study design such as study end points and LC n-3 PUFA dose or dietary advice given. Interestingly, in a prospective examination of the association between intake of fish and LC n-3 PUFA on risk of CVD and total mortality among 5103 female nurses with diagnosed T2DM 362 incident cases of CVD were documented between 1980 and 1996 (108). The subjects who consumed fish at least one to three times per month were found to have a 40% lower risk of developing CVD compared with those who ate fish less than once per month. However, subjects who ate fish five or more times per week were reported to experience a 64% reduction in CVD compared with those who ate fish less than once monthly (108). In contrast, a population study of 36,000 Iowa women (between 55 and 69 years of age) who were not diabetic and were monitored over 11 years has shown that development of T2DM is positively associated with LC n-3 PUFA (109). However, after adjustment for other dietary fat it was found that only vegetable fat is related to T2DM risk and appears protective. LC n-3 PUFA have been shown to lower TAG levels in subjects with T2DM or hypertriglycerolaemia. Supplementation with 1.8 g LC n-3 PUFA/d for 2 months in thirty-four patients with T2DM being treated with anti-diabetic drugs has been reported to reduce TAG levels; however, HDL-cholesterol levels increase (110).

Some animal studies seem to suggest that LC n-3 PUFA may affect muscle, liver and adipose tissue differentially within the insulin-resistant environment, which may reflect some of the inconsistent data observed. However, there is also some conflict in relation to the positive effects of LC n-3 PUFA in animal models of insulin resistance. Substitution of fish oil for SFA or MUFA or LC n-3 PUFA, in HFD (60% energy as fat) over 3 weeks in rats has been shown to completely prevent liver and muscle insulin resistance induced by the diets (111). An HFD containing LC n-3 PUFA or LC n-6 PUFA given to Wistar rats induces hyperglycaemia and hyperinsulinaemia, signs of insulin resistance (112). The HFD enriched with LC n-3 PUFA was shown to maintain GLUT4 content, insulin receptor, IRS-1 tyrosine phosphorylation and phosphatidylinositol 3-kinase activity in muscle but not in the liver. Furthermore, no change was found in GLUT4 and leptin mRNA within adipose tissue, as compared with a decrease when enriched in LC n-6 PUFA (111). In a model of the metabolic syndrome induced in rats an increase was found in body weight, systolic blood pressure, serum insulin, total lipids, TAG, cholesterol, NEFA, LDL, total proteins, albumin and serum TNFα (113). After fish oil administration rats with metabolic syndrome were shown to have reduced blood pressure, serum insulin, TAG, cholesterol and NEFA (113). However, no change was found in TNFα concentration or fat accumulation, which seems counterintuitive. The exact composition of the HFD and the amount of LC n-3 PUFA, specifically EPA and DHA, within the diet as well as the LC n-6 PUFA:LC n-3 PUFA may be a relevant factor in some of the conflicting information observed in these studies. In view of the recent evidence of pro-inflammatory macrophage infiltration into obese adipose tissue it will be of great interest to establish whether dietary fatty acid composition and LC n-3 PUFA:LC n-6 PUFA can affect (a) macrophage accumulation in adipose and (b) the phenotypic status of infiltrating macrophages (i.e. M1 or M2 polarisation).

**Conclusion**

Obesity produces a state of chronic low-grade inflammation with increased infiltration of macrophages into the adipose tissue. These ATM have been shown both in vivo and in vitro to production pro-inflammatory cytokines such as TNFα, IL-6 and MCP-1. These cytokines and chemokines induce and enhance the activation of the mitogen-activated protein kinases (c-Jun N-terminal kinase and extracellular signal-regulated kinase) and the activation of transcription factors such as NF-κB causing both the down-regulation and decreased activation of insulin signalling proteins (GLUT4 and IRS-1), which blocks insulin action and causes a state of insulin resistance. The adipocytes also become more pro-inflammatory with increased secretion of pro-inflammatory cytokines from the adipocytes. LC n-3 PUFA have been shown to influence gene expression (PPARγ and NF-κB) and eicosanoid production, reducing pro-inflammatory cytokine production from many different cells including the macrophage. However, the exact mechanisms of the interaction between macrophages and adipocytes and the effects of LC n-3 PUFA individually and in combination on both cell type need to be explored. Furthermore, much of the information in relation to LC n-3 PUFA protection in a T2DM environment remains in part conflicting and hypothetical. A clearer understanding of the effects of LC n-3 PUFA on muscle, liver and adipose tissue biology and insulin resistance within T2DM needs to be obtained.

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