Nutrient regulation of inflammatory signalling in obesity and vascular disease

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Abbreviations:

A20, TNFα-induced protein 3; AdipoR, adiponectin receptor; AGE, advanced glycation end product; Akt, protein kinase B; AMPK, AMP-activated protein kinase; AP-1, activator protein-1; APPL1, adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper-1; ASK1, apoptosis signal-regulating kinase 1; ATF6, activating transcription factor-6; ATM, adipose tissue macrophage; BCAA, branched chain amino acids;cIAP, cellular inhibitor of apoptosis protein;CR, caloric restriction; DAG, *sn*-1,2-diacylglycerol;DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ER, endoplasmic reticulum; eNOS, endothelial NO synthase; FA, fatty acid; FA-CoA, fatty acyl-CoA; FFAR1, free fatty acid receptor-1 (also known as GPR40); FFAR4, free fatty acid receptor-4 (also known as GPR120) ; GFAT1, glutamine:fructose-6-phosphate amidotransferase 1; GPCR, G-protein-coupled receptor; ICAM-1, intercellular adhesion molecule-1; IκB, inhibitor of NF-κB; IKK, IκB kinase; IL, interleukin; IL-1R, IL-1 receptor; IL-1RAcP, IL-1R accessory protein; IL-6R, IL-6 receptor; IRAK, IL-1R-associated kinase; IRE1α, inositol-requiring enzyme-1α; JAK, Janus kinase; JNK, c-Jun N-terminal kinase; LDL, low density lipoprotein; LOX-1, lectin-like oxidised LDL receptor-1; LPS, lipopolysaccharide;

LUBAC, linear ubiquitin chain assembly complex; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant protein-1; mTOR, target of rapamycin; mTORC, mTOR complex; MyD88, myeloid differentiation primary response gene 88; NF-κB, nuclear factor-κB; NLRP3, nucleotide oligomerization domain, leucine-rich repeat and pyrin domain-containing protein 3; NOX, NADPH oxidase; PERK, protein kinase RNA-like ER kinase; PI3K, phosphatidylinositol-3-kinase; PKC, protein kinase C; PKD, protein kinase D; PP2A protein phosphatase 2A; PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated FA; PVAT, perivascular adipose tissue; RAGE, receptor for AGE; RIP1, receptor-interacting serine/threonine-protein kinase 1; ROS, reactive oxygen species; SFA, saturated FA; Sp1, specificity protein-1; STAT, signal transducer and activator of transcription; T2DM, type 2 diabetes mellitus; TAB1, TAK1 and MAP3K7-binding protein 1; TAK1, TGFβ-activated protein kinase; TBK1, TANK-binding kinase 1; TLR4, Toll-like receptor-4; TNFα, tumour necrosis factor-α; TNF-R1, TNF receptor-1; TRADD, TNFR1-associated death domain protein; TRAF, TNF receptor-associated factor; TSC2, tuberous sclerosis complex-2.

SUMMARY

Despite obesity and diabetes markedly increasing the risk of developing cardiovascular disease, the molecular and cellular mechanisms that underlie this association remain poorly characterised. In the last twenty years it has become apparent that chronic, low-grade inflammation in obese adipose tissue may contribute to the risk of developing insulin resistance and type 2 diabetes. Furthermore, increased vascular pro-inflammatory signalling is a key event in the development of cardiovascular diseases. Overnutrition exacerbates pro-inflammatory signalling in vascular and adipose tissues, with several mechanisms proposed to mediate this. In this article, we review the molecular and cellular mechanisms by which nutrients are proposed to regulate pro-inflammatory signalling in adipose and vascular tissues. In addition, we examine the potential therapeutic opportunities that these mechanisms provide for suppression of inappropriate inflammation in obesity and vascular disease.

INTRODUCTION

Cardiovascular diseases are the leading cause of morbidity and mortality in people with type 2 diabetes mellitus (T2DM). It is now clear that obesity, T2DMand cardiovascular disease are associated with chronic low-grade inflammation manifesting as increased systemic levels of pro-inflammatory cytokines, particularly tumour necrosis factor-α (TNFα), interleukin-1β (IL-1β) and IL-6 [1-4].

Inflammation within the vascular wall and endothelial dysfunction are fundamental components of atherosclerotic vascular disease. Atherosclerosis is initiated by increased recruitment of circulating monocytes to localised areas of dysfunctional vascular endothelium, leading to development of atheromatous plaques [2]. Indeed, the inflammatory nature of atherosclerotic plaques was recognised by Rudolf Virchow in the mid-19th century, yet it required the development of monoclonal antibodies more than a century later to identify that foam cells within plaques were largely derived from leukocytes [2].In the last few decades, two key observations have highlighted the role of adipose tissue in regulating systemic inflammation. Firstly, adipose tissue has been demonstrated to act in an endocrine and paracrine manner by releasing a plethora of bioactive molecules, termed adipocytokines, that have pro-inflammatory and anti-inflammatory functions [5]. Secondly, increased macrophage infiltration was observed in obese adipose tissues of mice and humans [6,7]. This has given rise to the concept of metainflammation, whereby chronic overnutrition leads to increased pro-inflammatory signals derived from obese, dysfunctional adipose which sustains a chronic, low-grade inflammation in several tissues and may contribute to increased risk of T2DM [3,5]. The associations between overnutrition and inflammation demonstrate that nutrient signalling mechanisms influence inflammatory signalling both directly and indirectly in adipose and vascular tissues. In this review, we will examine the molecular mechanisms by which nutrient signals are perceived by adipose and vascular tissues to modulate inflammatory signalling pathways.

The key role of adipose tissue in overnutrition-related metabolic dysfunction

Caloric excess increases adipose tissue mass through the increased storage of triglyceride derived from either dietary triglycerides or carbohydrates. In the latter case, fatty acids (FAs) generated from excess circulating glucose are esterified to triglycerides by *de novo* lipogenesis. While white adipocytes ensure healthy storage

of excess nutrients, sustained nutrient overload can eventually exceed the capacity of hypertrophic adipocytes to store further triglycerides [8,9]. Consequently, ectopic triglyceride storage occurs in other tissues, including the liver and pancreas, leading to insulin resistance, T2DM and the complications associated with T2DM, including macrovascular and microvascular disease [9,10]. Pathological adipose tissue expansion due to sustained overnutrition also results in adipocyte fibrosis and death, with a dramatic increase in numbers of ATMs (adipose tissue macrophages) due to proliferation of existing macrophages and recruitment of monocytes[11,12]. Furthermore, overnutrition promotes the polarisation of ATMs towards a pro-inflammatory (M1) phenotype, altering the secretory profile of adipocytes, immune cells and other cell types within adipose tissue, further exacerbating insulin resistance (Figure 1)[11,12].Substantial evidence indicates that this metainflammation directly contributes to the development of insulin resistance and T2DM [11-13]. Furthermore, several existing anti-hyperglycaemic drugs used to treat T2DM including thiazolidinediones, metformin, SGLT2 inhibitors, incretin mimetics and insulin itself reduce inflammatory signalling[14]. Despite this, the precise mechanisms by which obesity-associated metainflammation is triggered remain poorly understood.

Inflammation in vascular disease

Endothelial dysfunction and increased inflammation within the vascular wall are key components of atherogenesis. Endothelial dysfunction due to dyslipidaemia, increased pro-inflammatory signalling, increased reactive oxygen species (ROS) and reduced nitric oxide (NO) bioavailability contributes to the proliferation of smooth muscle cells, recruitment of circulating monocytes and their subsequent differentiation into macrophages and foam cells that further exacerbates the pro-inflammatory environment (Figure 1)[2,15]. Indeed, the CANTOS trial using the IL-1β-sequestering antibody canakinumab provided proof-of-principle for inflammation being a causal component of atherogenesis [16].Given the association between increased cardiovascular disease risk and obesity, overnutrition has been proposed to promote endothelial dysfunction, vascular inflammation and atherogenesis. Indeed, as discussed further in this review, nutrients have direct actions on vascular tissue inflammationandsignals from dysfunctional metabolic tissues including adipose tissue can also impact vascular inflammation. In addition,

most blood vessels are surrounded by perivascular adipose tissue (PVAT) that regulates vascular function, releasing substances that maintain vascular health. As with other adipose tissue depots, PVAT becomes dysfunctional in obesity, with increased pro-inflammatory signalling that can directly influence the underlying blood vessels and impair vascular function [17,18]. Finally, multiple studies have demonstrated that insulin resistance within vascular tissues also plays an important role in the progression of atherosclerosis [19]. As systemic insulin resistance is also a risk factor for the onset and progression of hypertension and dyslipidaemia,insulin resistant individuals often, therefore, exhibit multiple risk factors that contribute to vascular inflammation and the progression of atherosclerosis [20].

PRO-INFLAMMATORY SIGNALLING PATHWAYS

As mentioned above, both obesity and vascular disease are associated with increased systemic levels of TNFα, IL-1β and IL-6. In addition, obesity is associated with reduced levels of the adipocytokine adiponectin, which has insulin-sensitising, anti-inflammatory and anti-atherosclerotic actions [21]. Furthermore, high circulating levels of adiponectin have been associated with reduced risk of coronary artery disease in certain populations [22,23]. Receptors for TNFα and IL-1β trigger pro-inflammatory effects by activating signalling pathways including the canonical NF-κB (nuclear factor-κB) and MAPK (mitogen-activated protein kinase) cascades responsible for triggering pro-inflammatory responses [24-26] (Figure 2). In contrast, IL-6 stimulates signalling pathways including activation of STAT (signal transducer and activator of transcription) proteins, which drive transcription of target pro-inflammatory genes [27] (Figure 2). On the other hand, adiponectin activates AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor (PPAR)-α which have anti-inflammatory actions [21,28]. These signalling pathways are considered in more detail below.

IL-1β receptor signalling

IL-1β is synthesised as a precursor (pro-IL-1β) in response to pro-inflammatory stimuli, including autocrine stimulation by IL-1 cytokines. Conversion of pro-IL-1β to active, secreted IL-1β is largely mediated by proteolysis by the NLRP3 (nucleotide oligomerization domain, leucine-rich repeat and pyrin domain-containing protein 3) inflammasome [13,29].Secreted IL-1β binds heterodimers of the IL-1 receptor (IL-1R)

with an accessory protein (IL-1RAcP). These recruit the adaptor molecule MyD88 (myeloid differentiation primary response gene 88) which results in the activation of multiple signalling pathways shown in Figure 2. Briefly, MyD88 interacts with IL-1R-associated kinase (IRAK)-4 and IRAK1, which recruit and activate the E3 ubiquitin ligase TRAF6 (TNF receptor-associated factor 6). Polyubiquitylation of downstream components promotes the recruitment and activation of TAK1 (TGFβ-activated protein kinase) which subsequently activates the canonical NF-κB pathway to trigger induction of multiple pro-inflammatory genes (Figure 2) [25,30]. TAK1 is also a MAPK kinase kinase, stimulating the activation of MAPKs including JNK (c-Jun N-terminal kinase) that can also stimulate transcription of pro-inflammatory mediators via activation of transcription factors including AP-1 (activator protein-1) (Figure 2) [31,32].The IL-1β signalling pathway is very similar to that engaged by stimulation of TLR4 (toll-like receptor-4), a pattern recognition receptor that is activated by lipopolysaccharide (LPS) from microorganisms, which also stimulates MyD88-IRAK-TRAF6-TAK1 signalling (Figure 2) [26,31]. Many of the pro-inflammatory effects of IL-1R and TLR4 stimulation are mediated by NF-κB and JNK activation, yet both receptor complexes also engage other signalling pathways, details of which have been reviewed extensively elsewhere [25,26,30].

TNFα receptor signalling

TNFα mediates its effects via the ubiquitously expressed TNF-R1 (TNF receptor-1) or TNF-R2 which exhibits more restricted expression [33]. Activation of trimeric TNF-R1 complexes by TNFα results in recruitment of a multi-protein complex containing the adaptor protein TRADD (TNFR1-associated death domain protein), the protein kinase RIP1 (Receptor-interacting serine/threonine-protein kinase 1), and the E3 ubiquitin ligases TRAF2, TRAF5, cIAP1 (cellular inhibitor of apoptosis protein-1), cIAP2 and LUBAC (linear ubiquitin chain assembly complex). This complex then recruits and activates TAK1 and IKK (inhibitor of NF-κB [IκB] kinase), thereby stimulating activation of NF-κB and pro-inflammatory MAPKs including JNK (Figure 2) [34]. TNFα stimulation therefore increases transcription of pro-inflammatory genes by similar mechanisms to IL-1β. For more details of TNFα signalling pathways, the reader is directed to a recent review [34].

IL-6 receptor signalling

IL-6 signalling requires IL-6 binding to an IL-6 receptor (IL-6R) followed by interaction with dimeric complexes of the ubiquitously expressed signal transducer receptor gp130. Altenatively, IL-6 can also signal by binding to a soluble IL-6R (sIL-6Rα) before the resulting complex binds and activates gp130 (Figure 2) [27,35].Either mode of signalling activates receptor-bound JAKs (Janus kinases) which phosphorylate specific cytosolic Tyr residues of gp130. These phosphotyrosine residues act as recruitment sites for STAT transcription factors (mainly STAT3) [27,35]. Recruited STAT3 proteins are subsequently phosphorylated on Tyr705 by gp130-bound JAK and homodimerise. STAT3 is also phosphorylated on Ser727 by several protein kinases including mTOR (mammalian target of rapamycin)[35,36]. STAT3 dimers bind specific gene promoters to initiate target gene transcription. Phosphorylated STAT3 has also been reported to localise to mitochondria, regulating the electron transport chain to limit generation of ROS [37]. Phosphorylated gp130 can also signal independent of STATs by recruiting other signalling modules, which are reviewed elsewhere [35] (Figure 2).

ADIPONECTIN SIGNALLING

Adiponectin is synthesised almost exclusively by mature adipocytes and forms multimeric complexes [21,38]. A truncated globular domain form of adiponectin has also been reported to circulate [39], although multimeric adiponectin may be more active *in vivo* [38]. Adiponectin signals through two cell surface receptors, AdipoR1 and AdipoR2 [21,22,40]. Furthermore, multimeric adiponectin has been reported to accumulate in tissues including the vascular endothelium through an interaction with T-cadherin [21,41]. AdipoR1 and AdipoR2 signalling involves the intracellular binding partner APPL1 (adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper-1), which is required for adiponectin-mediated stimulation of AMPK[42,43], a key regulator of energy metabolism that has anti-inflammatory actions, described later in this review. AdipoR1 or AdipoR2 also have ceramidase activity and increase PPARα activity, thereby improving insulin sensitivity by removing ceramides that impair insulin signalling and promoting FA oxidation and energy expenditure [28,44].

LIPID METABOLISM, OVERNUTRITION AND LIPOTOXICITY

As described before, when sustained nutrient overload exceeds the storage capacity of hypertrophic adipocytes, ectopic triglyceride storage occurs in other tissues, which subsequently contributes to insulin resistance. The anti-lipolytic actions of insulin are lost in insulin-resistant adipocytes, leading to high plasma FA concentrations. This increased concentration of harmful lipids that impairs cellular homeostasis and disrupts tissue function is known as lipotoxicity [8,9].

Early studies investigating the relationship of dietary FAs with cardiovascular disease indicated that populations with high consumption of saturated FAs (SFAs) were associated with increased cardiovascular disease mortality, whereas high consumption of *ω*-3 polyunsaturated FAs (PUFA) was associated with reduced cardiovascular disease mortality [45]. Furthermore, dietary FA composition modulates the concentration of circulating cholesterol and triglycerides in lipoproteins [46]. These observations prompted recommendations on dietary FA intake for primary and secondary prevention of cardiovascular diseases [47]. Excess low density lipoprotein cholesterol is a well-known risk factor for atherosclerosis, due to the key role cholesterol and modified cholesterol play in the development of foam cells [48], yet significant research has also investigated the effects of long chain SFAs, such as palmitate on inflammatory signalling pathways in vascular and adipose cells.

The potential mechanisms by which FAs can influence inflammatory signalling fall into twocategories. Firstly, FAs can act directly as ligands for receptors including PPARs and G-protein-coupled receptors (GPCRs) that regulate inflammatory signalling [49-51]. Secondly, metabolites of FAs such as *sn*-1,2-diacylglycerol (DAG), ceramides and fatty acyl-CoA (FA-CoA) influence signalling proteins including protein kinase C (PKC), protein kinase D (PKD), protein phosphatase-2A (PP2A) and TLR4 that promote inflammatory signalling whilst impairing insulin signalling (Figure 3)[52-54]. Each of these is discussed in more detail later in this review.

Nuclear lipid receptors and inflammation

FAs activate the PPAR family of nuclear receptors that are well-established regulators of lipid metabolism and mitochondrial biogenesis [50,51]. In addition, PPARs have defined roles in immune cells, regulating their differentiation and function [50,51]. Activation of PPARγ or PPARα in macrophages is associated with increasing polarisation toward the alternatively activated M2 phenotype, thereby

having anti-inflammatory actions [55,56]. Furthermore, stimulation of adipocyte PPARγ increases adiponectin expression and promotes safe lipid storage by adipogenesis, thereby reducing lipotoxicity and improving insulin sensitivity [57]. PPARα stimulation attenuates dyslipidaemia and both PPARα and PPARγ agonists have been reported to suppress cytokine-stimulated expression of adhesion molecules and pro-atherogenic chemokines in vascular endothelial cells [51,58,59]. Although these studies demonstrate that stimulation of PPARα/γ has anti-inflammatory actions, acting to suppress the detrimental actions of excess lipids, PPARγ activation also promotes foam cell formation by upregulating the scavenger receptor CD36, permitting greater modified LDL (low density lipoprotein) uptake [60].

GPCR lipid receptors and inflammation

Over the last few decades,de-orphanisation efforts have revealed several GPCRs where metabolites are the endogenous agonists. Theseinclude FFAR1 and FFAR4 (free fatty acid receptors 1 and 4; otherwise known as GPR40 and GPR120), which are activated by long chain FAs [49,61].Activation of FFAR4, which is highly expressed in activated macrophages and adipose tissue, by *ω*-3 unsaturated FAs was reported to suppress pro-inflammatory TLR4 and TNFα signalling via inhibition of TAK1 [62]. In that study, ligand-bound FFAR4 was demonstrated to internalise leading to association of β-arrestin with TAB1 (TAK1 and MAP3K7-binding protein 1), blocking the association of TAB1 with TAK1 and thereby supressing downstream activation of IKK and JNK [62]. In the same study, supplementation with *ω*-3 unsaturated FAs reduced macrophage infiltration of adipose tissues in high fat diet-fed mice in a FFAR4-dependent manner [62]. Indeed, FFAR4 may also increase adipogenesis [61], such that it acts in a similar manner to PPARγ by suppressing inflammation and increasing triglyceride storage capacity.

On the other hand, global deletion of FFAR1 in mice had no effect on adiposity or insulin sensitivity [63,64]. In endothelial cells, however, FFAR1 activation has been reported to have pro-inflammatory actions, increasing ICAM-1 (intercellular adhesion molecule-1) expression and IL-6 secretion [65,66]. It therefore remains unclear the extent to which direct signalling via FFARs underlie or modulate the actions of FAs on inflammatory signalling pathways, although the data from FFAR4-decificient mice suggest an important effect on metainflammation [62].

Effects of lipid metabolites on inflammation: DAG and PKC

PKC isoforms are a family comprised of three subgroups, where the conventional and novel PKCs are activated by DAG [53]. In addition, PKD is a family of serine/threonine protein kinases that are also sensitive to DAG and act as downstream effectors of PKC in certain systems (Figure 3) [53]. As prolonged exposure to palmitate increases intracellular synthesis of DAG, palmitate-stimulated PKC activation has been demonstrated in isolated arteries, vascular smooth muscle cells, endothelial cells and adipocytes [67-70]. Intracellular DAG levels are also increased by hyperglycaemia (Figure 4), thereby activatingDAG-sensitive PKC isoforms in vascular cells and adipocytes [71-73]. In hyperglycaemia, ROS have also been reported to stimulate PKC isoforms in a DAG-independent manner in vascular cells [74,75]. Consequently many studieshave investigated the role of PKC in obesity, atherogenesis and insulin resistance, utilizing PKC isoform-selective inhibitors or genetic downregulation of specific PKC isoforms and have been reviewed elsewhere [53,76].

In the context of inflammation, PKCs have an important role in multiple aspects of both innate and adaptive immunity [77], whereas PKD has been implicated in NRLP3 inflammasome activation in bone marrow-derived macrophages [78]. In 3T3-L1 adipocytes, disruption of PKC activity suppressed palmitate-stimulated activation of JNK, IKK and IL-6 expression [69,79,80]. In endothelial cells, high glucose increased PKC activity whereas PKC inhibition attenuated high glucose-induced expression of the adhesion molecules ICAM-1 and E-selectin and subunits of NOX2 (NADPH oxidase complex-2) [81,82] as well as high glucose or palmitate-stimulated ROS synthesis in endothelial and vascular smooth muscle cells (Figure 3) [67]. These studies suggest PKC activation contributes to lipid and hyperglycaemia-induced vascular inflammation and ROS production (Figure 4). Many of these studies have, however, used small molecule inhibitors of PKC, which exhibit various levels of selectivity [83,84], such that caution should be taken when assigning PKC-dependence of effects. Some potential substrates of PKC have been identified in the context of inflammation, including components of NOX2 and NOX5 [85,86], eNOS (endothelial NO synthase) at the inhibitory Thr495 site [87] and intermediates in cytokine signalling pathways including TRAF2 and the tyrosine kinase Syk (Figure 3) [88,89]. These effects would impair NO synthesis, activate TAK1/IKK and increase Syk-mediated NF-κB activation. In contrast, PKCδ has been reported to inhibit

NF-κB activation by phosphorylating the deubiquitinase A20(TNFα-induced protein 3) in bone marrow-derived macrophages [90]. Therefore, PKC activation contributes to increased NF-κB and JNK activation during nutrient overload through several potential mechanisms(Figure 3) that are not necessarily exclusive.

Lipotoxicity and ceramide biosynthesis

In addition to DAG synthesis, high palmitate concentrations also lead to ceramide biosynthesis, which has been associated with insulin resistance and NRLP3 inflammasome activation (Figure 3) [13,54]. Ceramide accumulation is also stimulated by TNFα, whereas adiponectin receptors have been reported to exhibit ceramidase activity, thereby reducing ceramide levels [28,44]. Ceramides were first demonstrated to induce insulin resistance in 3T3-L1 adipocytes, reducing recruitment of Akt (protein kinase B) to the plasma membrane upon insulin stimulation and increasing PP2A activity, leading to Akt inactivation [54,91]. As Akt phosphorylates and activates eNOS, ceramides also inhibit NO production in endothelial cells [92], which would be predicted to suppress the anti-inflammatory actions of NO in the vessel wall. Lipotoxicity-induced increases in ceramide levels therefore reduce adipocyte insulin sensitivity and endothelial NO synthesis, which may further increase pro-inflammatory signalling in both tissues.

Regulation of TLR4 signalling by FAs

As detailed earlier, activation of TLR4 stimulates a signalling cascade that activates NF-κB and pro-inflammatory MAPKs including JNK (Figure 2) [26,31]. Deletion of TLR4 prevented high fat diet-induced insulin resistance and endothelial dysfunction in mice as well as palmitate-induced IKK activation in aortic explants, indicating a direct role of TLR4 in the pro-inflammatory actions of overnutrition [93]. This stimulatory effect on TLR4 is most likely intracellular, with recent evidence indicating it requires esterification of FAs to FA-CoA (Figure 3)[52].

Anti-inflammatory actions of polyunsaturated FAs

PUFA, in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been reported to have largely anti-inflammatory actions in adipose and vascular tissues, in marked contrast to the actions of SFAs [94,95]. The mechanisms by which these anti-inflammatory actions occur are, however, not well-characterised.

As described above, FFAR4 activation by *ω*-3 PUFA has been demonstrated to suppress TLR4 and TNFα signalling via inhibition of TAK1 activation [62]. The effects of *ω*-3 PUFA may also include activation of PPARα and/or PPARγ [96,97]. Furthermore, lipoxygenase metabolism of EPA and DHA leads to the production of anti-inflammatory resolvins and protectins which promote resolution of inflammation [98]. In obese mice, levels of resolvins have been reported to be suppressed, whereas their replacement reduced adipose tissue inflammation and improved insulin sensitivity, indicating that the balance of pro-inflammatory saturated FA and anti-inflammatory PUFA signalling is important in obesity-induced inflammation [99]. It should also be noted that although many of the actions of PUFA in cultured cells or rodent models have indicated a beneficial effect on inflammation and cardiovascular risk, human interventions have yielded inconsistent results [100].

HYPERGLYCAEMIA & GLUCOTOXICITY

Suppression of prolonged hyperglycaemia, a key feature of diabetes, has been consistently shown to reduce microvascular disease outcomes, although many intervention studies have failed to demonstratereduced macrovascular disease outcomes [101]. The damage due to hyperglycaemia, referred to as glucotoxicity, may be due to several pathways, including increased polyol pathway activation and advanced glycation end product (AGE) synthesis, increased hexosamine pathway flux, activation of PKC and production of ROS (Figure 4) [102,103]. All these pathways have been reported to influence inflammatory signalling as discussed further below.

Altered glucose metabolism in hyperglycaemia

Glycation, the nonenzymatic covalent modification of proteins by glucose or fructose metabolitesis mediated by a complex series of sequential reactions between glycolytic intermediates and amino groups of proteins, leading to the synthesis of AGE [103]. Methylglyoxal, formed from triose phosphate glycolytic intermediates, has been proposed to account for most hyperglycaemia-induced AGE adducts [104]. Once formed, AGE are not easily metabolised and accumulate in those with sustained hyperglycaemia. Furthermore, in some cells excess glucose can be converted to fructose *via* sorbitol by the polyol pathway rather than be metabolised *via* hexokinase to glucose-6-phosphate (Figure 4). Further metabolism of fructose

generated by the polyol pathway can contribute to the formation of AGEs [105]. Increased intracellular sorbitol concentrations have been reported in cultured human endothelial cells exposed to high glucose [106,107], whereas enhanced aldose reductase activity, which catalyses the initial step of this pathway, has been reported in human monocyte-derived macrophages under similar conditions [108]. There are few published reports examining the polyol pathway in adipocytes or adipose tissue, although increased methylglyoxal levels have been reported in3T3-L1 adipocytes exposed to high culture glucose in the absence of any changes in sorbitol levels [109].

In addition to polyol pathway flux and AGE synthesis, hyperglycaemia can also stimulateflux from fructose-6-phosphate through the hexosamine pathway, increasing post-translational modification of proteins by *O*-GlcNAcylation (Figure 4). Increased *O*-GlcNAcylation of proteins has been widely reported in cultured human endothelial cells exposed to high glucose, as well as cultured human adipocytes, which may contribute to altered inflammatory signalling pathwaystatus [110-112]. The effects of AGE and *O*-GlcNAcylation on adipose tissue and vascular inflammation are discussed further below.

AGE signalling and inflammation

AGEs bind several cell surface receptors including the receptor for AGE (RAGE), scavenger receptors, galectin-3 and LOX-1 (lectin-like oxidised LDL receptor-1) [103,113]. RAGE is likely to mediate most biological actions of AGEs but can also be activated by members of the pro-inflammatory S100/calgranulin family of alarmin proteins [103,114]. Furthermore, soluble RAGE (sRAGE) isoforms act as decoy receptors, attenuating RAGE signalling [115,116]. The initial signalling pathways involved after ligand binding of RAGE are poorly characterised but lead to increased ROS synthesis and MyD88-dependent signal transduction as described for IL-1β and TLR4 agonists, with substantial evidence of crosstalk between RAGE and TLR signalling [117-120]. In vascular cells, increased RAGE signalling stimulates pro-inflammatory JNK and NF-κB signallingleading to increased cytokine, chemokine and adhesion molecule synthesis (Figure 4)[121-123]. Indeed, the importance of AGE/RAGE signalling in atherosclerosis has been demonstrated in mice with a homozygous deletion of RAGE [124]. Taken together, these studies clearly demonstrate increased RAGE activation contributes to the pro-inflammatory actions

of sustained hyperglycaemia in vascular tissues in mice. Similarly, AGE/RAGE signalling has been reported to increase pro-inflammatory signalling, ROS production, adipocyte hypertrophy and insulin resistance in 3T3-L1 adipocytes and mice [125-127]. As circulating levels of inhibitory sRAGE are inversely associated with obesity in humans [128-130], this will further exacerbate AGE/RAGE signalling (Figure 4). Therefore, increased RAGEactivationduring hyperglycaemia and obesity likely contributes to inflammatory signalling in both adipose and vascular tissues, particularly the microvasculature in humans.

O-GlcNAcylation and inflammation

As described above, increased *O*-GlcNAcylation of proteins has been demonstrated in cultured human endothelial cells and adipocytes exposed to high glucose [110-112].In the context of inflammation, a variety of specific substrates for *O*-GlcNAcylation have been reported, including IKKβ and the TAK1 binding protein TAB1in various cell types (Figure 4) [131,132].A few potentialsubstrateshave been identified in vascular cells, including increased *O*-GlcNAcylation of the RelA/p65 subunit of NF-κB in vascular smooth muscle cells, leading to increased transcriptional activity in response to high glucose [133]. In endothelial cells, *O*-GlcNAcylation of the transcription factor Sp1 (specificity protein-1) and eNOS has been proposed to underlie hyperglycaemia-induced increases inICAM-1expressionand suppression of NO synthesis [134,135].It should be noted that *O*-GlcNAcylation of proteins may also attenuate pro-inflammatory signalling, including TNFα-stimulated NF-κB activation in vascular smooth muscle cells due to *O*-GlcNAcylation of A20 [136]. Similarly, *O*-GlcNAcylation of STAT3 in macrophages was proposed to inhibit transcriptional activity [137]. These studies indicate that increased *O*-GlcNAcylation of pro-inflammatory and anti-inflammatory proteins may occur in response to hyperglycaemia, particularly in vascular cells, yet the functional significance and extent of these post-translational modifications remains to be established.

OVERNUTRITION, ENDOPLASMIC RETICULUM STRESS, OXIDATIVE STRESS AND INFLAMMATION

The endoplasmic reticulum (ER) is a major hub of lipid biosynthesis and esterification, and the ER is dysregulated under conditions of overnutrition and by

AGE, leading to ER stress in adipose and vascular tissues [138-141]. ER stress is characterised by the activation of three ER-anchored transmembrane receptors, IRE1α (inositol-requiring enzyme-1α), PERK (protein kinase RNA-like ER kinase) and ATF6 (activating transcription factor-6) [142].Increased ER stress has been proposed to activate pro-inflammatory signalling byseveral mechanisms. Inhibition of IRE1α reduced activation of the NRLP3 inflammasome in peripheral blood mononuclear cells, suggesting ER stress increases NRLP3 inflammasome activation[143]. Furthermore, ER stress has been associated with IKK/NF-κB pathway activation, *via* a mechanism that may involve maintenance of basal IKK activity by IRE1α and PERK [144]. Activated IRE1α has also been reported to form a complex with TRAF2 that can lead to activation of NF-κB as well as recruitment of ASK1 (apoptosis signal-regulating kinase 1), which can subsequently activate JNK [145].Indeed, ER stress is intimately linked with oxidative stress, as ROS signals can induce ER stress, and ER stress can generate ROS, further exacerbating pro-inflammatory signalling [142,146].

Increased ROS production has been reported in both adipose and vascular tissues in response to hyperglycaemia/RAGE signalling and high palmitate concentrations (Figures 3 & 4) [67,147-149]. Significant evidence links overnutrition to oxidative stress and subsequent inflammation in which ROS can be generated by several mechanisms including increased activity of some NOX complexes, uncoupled eNOS, mitochondrial respiration, ER stress and reduced antioxidant capacity [20]. Evidence thatROS synthesised under conditions of overnutrition influences inflammatory signalling includes use ofthe antioxidantapocyanin, which reduced high fat diet-induced adipose tissue inflammation including NF-κB activity, expression of TNFα and the pro-inflammatory chemokine MCP-1 (monocyte chemoattractant protein-1) [150]. Furthermore, excess glucose and palmitate increased ROS generation and MCP-1 expression in a NOX4-dependent manner in 3T3-L1 adipocytes [151], whereas deletion of NOX4 in mouse adipocytes protected against high glucose and palmitate-stimulated IL-6, IL-1β and MCP-1 expression [152]. Indeed, in the same study, adipocyte-specific deletion of NOX4 activity attenuated the initial increases in adipose tissue MCP-1, TNFα mRNA and macrophage infiltration during a high calorie diet [152].Similar data have been reported in cultured endothelial cells, where knockdown of NOX4 attenuated palmitate-stimulated phosphorylation of IκB (inhibitor of NF-κB) and IL-6 secretion,

indicating a role for NOX4-derived superoxide in palmitate-stimulated NF-κB activation [153].It should be noted, however, the role of NOX4 is unclear in cardiovascular disease, as it has also been shown to have vasoprotective actions through synthesis of H_2O_2 [154]. In endothelial cells, palmitate stimulated superoxide production was reported to be TLR4, MyD88 and IRAK1-dependent, suggesting that palmitate activates TLR4 signalling, stimulating ROS production that exacerbates NF-κB signalling [153]. Similar observations have been made in the THP-1 human monocytic cell line [155]. Superoxidealso sequesters NO, thereby reducing the anti-atherogenic, anti-inflammatory actions of NO [156]. Taken together, these studies clearly indicate that overnutrition-stimulated oxidative stressin vascular and adipose tissues makes a significant contribution to the inflammation underlying the development of insulin resistance and atherosclerosis, yet the mechanisms by which ROS are generated, and the particular ROS involved remain to be fully characterised.

REGULATION OF AMPK AND mTOR BY NUTRIENTS – COORDINATING ENERGY SUPPLY WITH INFLAMMATORY SIGNALLING

AMPKand mTOR are interlinked signalling pathways that sense nutrient availability and act to regulate cellular metabolism and growth [157]. AMPK acts as a cellular energy sensor, activated by an increase in the AMP:ATP ratio that occurs when nutrient levels fall due to reduced ATP synthesis or increased ATP utilization[157-161]. More recently, it has become apparent that glucose starvation and long chain FA-CoA can also activate AMPK by AMP-independent mechanisms (Figure 5) [162,163]. AMPK acts to stimulate ATP synthesis, inhibiting pathways that consume ATP such as lipogenesis, cholesterol synthesis, protein synthesis and gluconeogenesiswhilst stimulating catabolic pathways that generate ATP, such as FA oxidation, GLUT4-mediated glucose uptake and mitochondrial activity [160,161].

mTOR is a Ser/Thr-directed protein kinase which integrates signals from growth factors, including insulin, and nutrient sensors to maintain cellular homeostasis, existing in at least two distinct complexes termed mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) [157]. Amino acids induce translocation of mTORC1 from the cytoplasm to thelysosomal membrane via specific sensors which ultimately converge on a Ragulator multi-protein complex and Rag GTPases (Figure 5).Activation of mTORC1 by amino acids and growth factors leads to stimulation of

protein translation and cell growth [164]. AMPK phosphorylates the mTORC1 subunit Raptor and upstreamregulator TSC2 (tuberous sclerosis complex-2), leading to suppression of mTORC1 activity. AMPK and mTORC1 therefore act in opposition, activated by nutrient depletion and increased nutrient availability respectively (Figure 5) [157].

Suppression of AMPK activity by overnutrition

Reduced AMPK activity has been reported in many tissues of mice fed a high fat diet, including adipose tissue and the aortic endothelium [165-167]. Intriguingly, mice fed a high fat diet containing monounsaturated rather than SFAs exhibited increased adipose tissue AMPK activity [168], indicating that AMPK downregulation after a high fat diet is largely due to increased SFA concentrations. Furthermore, hyperglycaemia has been reported to inhibit AMPK in certain tissues and cell types, including muscle, liver and kidney [169,170]. Importantly, these findings have been translated to adipose tissue in man, where reduced AMPK activity has been reported to be associated with insulin resistance in morbidly obese people [171], whereas weight loss increases AMPK activity [172,173]. In vascular and adipose tissues it is less clear whether hyperglycaemia alone inhibits AMPK activity. Experimental hyperglycaemia has been reported to reduce AMPK activity in vascular smooth muscle cells and a macrophage cell line yet had no effect in cultured endothelial cells [174-176]. Despite this, AMPK inhibition has been reported when endothelial cells are cultured in high concentrations of both glucose and palmitate [177].

In contrast to the suppression of AMPK by overnutrition, long chain FA-CoA formed after FA transport into cells activate AMPK allosterically, thereby promoting FA oxidation [163].Activation of AMPK by long chain FA-CoA or low glucose therefore occurs under physiological conditions, whereas overnutrition inhibits AMPK (Figure 5). Although overnutrition may decrease the cellular AMP:ATP ratio, several other mechanisms may play a role in regulating AMPK under these conditions, including increased PKC-mediated inhibitory phosphorylation of AMPK and reduced adiponectin-mediated AMPK activation [42,43,178,179]. The important role of AMPK in nutrient metabolism has led to its proposal as a therapeutic target for conditions of dysfunctional metabolism, yet AMPK activation is also associated with anti-inflammatory and anti-atherosclerotic actions, discussed later in this review [159,161].

Stimulation of mTORC1 by overnutrition

Several studies have demonstrated mTORC1 is hyperactivated in metabolic tissues of obese and high-fat-diet fed rodents [180,181]. Furthermore, mTOR gene expression is upregulated in visceral fat of human subjects with obesity and insulin resistance [182]. Others, however, have reported reduced mTOR activity in adipocytes from patients with T2DM, associated with enhanced autophagy [183]. Severalmechanisms may account for hyperactivation of mTORC1signalling in the setting of overnutrition, includingsuppressed AMPK activity, activation by proinflammatory cytokines and elevated branched-chain amino acids (BCAAs), which trigger chronic activation of mTORC1[157,184-186] (Figure 5). Although interventional studies have shown that increasing dietary BCAA intake have beneficial effects on body composition and glucose homeostasis, potentially due to direct effects on mechanisms controlling satiety [187,188], increased fasting concentrations of circulating BCAAs are associated with increased risk of T2DM and cardiovascular disease in both animal models and humans [186,189]. Importantly, longitudinal studies have observed that elevated levels of circulating BCAAs are predictive of future insulin resistance or T2DM, suggesting a potential causative role [190,191]. In addition, studies aimed at identifying plasma metabolic signatures of visceral adiposity have shown a strong association with BCAA in the absence of any association with T2DM [192]. Thus, despite the beneficial effects of dietary BCAA supplementation, sustained dysregulation of circulating BCAA levels is indicative of insulin resistant and T2DM phenotypes.

Anti-inflammatory actions of AMPK

Early studies reported anti-inflammatory actions of the AMPK activator AICAR in human endothelial cells and adipose tissue [193-195]. In subsequent studies, AMPK-dependent anti-inflammatory actions were demonstrated *in vivo* in both vascular and adipose tissues [196-200].Indeed, AMPK is anti-atherogenic in atherosclerosis-prone hypercholesterolemic mice and rodent models of vascular injury [161], whilst also acting to suppresses macrophage differentiation and foam cell formation [201-203].

There are several mechanisms by which AMPK has been proposed to influence inflammation signalling. AMPK activation reduces NLRP3 inflammasome activation

in macrophages [168], potentially by reducing ER stress [204]. AMPK has been demonstrated to impair NF-κB activation in response to pro-inflammatory stimuli in multiple studies in vascular and adipose tissue cells [199,205-207].Several mechanisms have been proposed for the AMPK-mediated suppression of NF-κB, including direct phosphorylation and inhibition of IKKβand AMPK-mediated phosphorylation and inhibition of the transcriptional co-activator p300(Figure 6)[205,208]. In addition, AMPK activation inhibited IRAK4 phosphorylation in IL-1β-stimulated cells [199], suggesting AMPK attenuates IL-1β/LPS-mediated signalling upstream of NF-κB activation (Figure 6). Furthermore, AMPK stimulates NO synthesis [161], which has been demonstrated to inhibit endothelial NF-κB activity [209]. More recently, TANK-binding kinase 1 (TBK1), animportant component of innate immunity that also regulates autophagic signalling was shown to be required for AMPK-mediated inhibition of NF-κB [210]. AMPK activation may therefore attenuate NF-κB activation by more than one mechanism, depending on the stimulus and the cell or tissue type.

Given the effect ofAMPK on IL-1β-stimulated IRAK4 phosphorylation, it is perhaps unsurprising that AMPK activators also attenuate pro-inflammatory cytokine-stimulated phosphorylation of JNK and its upstream kinase MKK4 (Figure 6) [199,211]. In addition, studies in our groups demonstratedAMPK-mediated inhibition of IL-6-stimulated JAK-STAT signalling in endothelial cells and 3T3-L1 adipocytes [199,212] via direct inhibitory phosphorylation of JAK1 by AMPK (Figure 6) [212]. AMPK activation, therefore,utilises diverse mechanisms to rapidly suppress multiple pro-inflammatory signalling pathways in vascular and adipose tissues.

There are other, less direct mechanisms by which AMPK activation may suppress inflammatory signalling including reduced *O*-GlcNAcylation [213], ER stress [214] and ROS synthesis [215,216]. AMPK phosphorylates and inhibits GFAT1 (glutamine:fructose-6-phosphate amidotransferase 1) in endothelial cells, the rate-limiting enzyme in the hexosamine biosynthesis pathway, thereby reducing cellular *O*-GlcNAcylation (Figure 6) [217]. The mechanisms by which AMPK acts to reduce ROS are unclear, although reduced expression and translocation of NOX2 complexesto the plasma membrane and increased expression of antioxidant enzymes have been reported [215,216].

Given the suppression of AMPK in overnutrition, these studies highlight the multiple potential mechanisms by which reduced AMPK activity may contribute to

metainflammation during obesity and vascular disease. This hasfurther highlighted AMPK as a potential therapeutic target for suppressing metainflammation.

mTORC1 and inflammatory signalling

Pro-inflammatory cytokines involved in vascular disease, obesity and T2DM, including IL-1β and TNFα, activate mTORC1 via PI3K (phosphatidylinositol-3-kinase) (Figure5) [184,185]. While there is strong genetic evidence for critical roles of mTOR in the expansion and differentiation of T cell subsets [218], there is relatively little information on vascular inflammation. However, mTOR has been shown to enable a pro-inflammatory phenotype in vascular endothelial cells in part through sustaining TNFα-stimulated adhesion molecule induction [219].Another mechanism by which mTOR can influence inflammation is by phosphorylation of STAT3 at Ser727 [220]. STAT3 Ser727 phosphorylation ensures maximal transcriptional activation, and several reports indicate that optimal STAT3 activation requires a functional mTOR pathway [221,222]. Phosphorylation at this site may be particularly important for the recently described non-canonical role of STAT3 in maintaining mitochondrial integrity and suppressing production of reactive oxygen species in adipocytes and other cell types (Figure 2) [220,223]. The significance of these mechanisms for control of STAT3 function in other immune cell types and vascular endothelial cells remains to be explored.

A further consequence of mTOR activation is inhibition of autophagy. Basal autophagy may protect against atherosclerosis by limiting inflammation yet chronic suppression of autophagy, triggered by pro-atherogenic factors such as oxidative stress, inflammation and oxidized lipoproteins, may exacerbate atherosclerosis [224]. Inhibition of mTOR, therefore, represents one potential approach to normalise autophagic flux and limit vascular inflammation. This strategy is supported by the reduced macrophage accumulation in adipose tissue and suppressed pro-inflammatory gene expression in macrophage-specific Raptor deficient mice fed a high fat diet [225]. This was proposed to be due to enhanced Akt signalling and suppression of NF-κB and JNK pathways [225]. In contrast, targeted deletion of Raptor in adipocytes exacerbates adipose tissue inflammation due to oxidative stress and activation of the NLRP3 inflammasome despite reducing weight gain in response to high fat diet [226]. Therefore, any effects of mTOR inhibition on inflammatory mechanisms are likely to be cell type-specific.

NUTRIENT-REGULATED HORMONAL SIGNALLING AND INFLAMMATION

In addition to the direct actions of nutrients or nutrient metabolites on inflammation signalling, nutrients influence many signalling pathways via hormonal signalling, principally insulin, glucagon, adipocytokines and incretin hormones. As highlighted earlier in this review, insulin resistance is closely linked to pro-inflammatory signalling in obesity and insulin has been proposed to regulate both adipose and vascular tissue inflammation directly, such that overnutrition may also influence inflammation via insulin-mediated mechanisms.

Obesity is often associated with hyperinsulinaemia, and studies in humans have reported increased levels of TNFα, MCP-1, IL-6 and IL-8 in adipose tissue during a hyperinsulinaemic-euglycaemic clamp, with similar observations made in mice [227-231]. Indeed, a strong correlation has been reported between circulating insulin levels and MCP-1, IL-6, TNFα and IL-1β expression in adipose tissue in both humans and mice [231]. Furthermore, the insulin-stimulated increases in adipose tissue pro-inflammatory cytokine expression may be exacerbated by insulin resistance [229,232]. Taken together, these studies indicate that hyperinsulinaemia contributes to adipose tissue inflammation in obesity, which may exacerbate insulin resistance. In contrast, chronic insulin therapy was reported to decrease macrophage content in adipose tissue of obese, atherosclerosis-prone mice [233].

In vascular tissue, insulin has long been known to stimulate eNOS activity [234,235], suppressing ICAM-1 expression in a manner sensitive to NOS inhibition, whilst also inhibiting NF-κB activation and MCP-1 expression [236,237]. In contrast to these anti-inflammatory actions of insulin, excessive insulin signalling in the endothelium accelerates pro-atherogenic pro-inflammatory signalling including adhesion molecule expression, leukocyte adhesion and NF-κB activation [238-240]. Elegant mouse models of impaired endothelial insulin signalling have reinforced that endothelial insulin sensitivity is important for the maintenance of vascular health [241,242]. Indeed, mice in which vascular insulin sensitivity is enhanced also exhibit pro-atherosclerotic signalling [243]. These rodent studies,therefore,suggest that both impaired and excessive insulin signalling may exacerbate vascular inflammation and promote atherogenesis.

As described earlier in this review, circulating adiponectin concentrations are reduced in obesity, likely due to TNFα-mediated inhibition of expression [21].

Adiponectin has been reported to supress LPS-stimulated NF-κB activation, IL-6, TNFα and MCP-1 expression in 3T3-L1 adipocytes [244,245], suggesting it may have autocrine actions, yet most research has focussed on the actions of adiponectin on inflammation in leukocytes and vascular endothelial cells. Early studies demonstrated that adiponectin attenuated NF-κB activation and pro-inflammatory cytokine and adhesion molecule expression in endothelial cells and macrophages [246-248], also increasing IL-10 in macrophages, promoting their polarisation toward an anti-inflammatory phenotype [249,250]. Since then, significant numbers of studies have indicated that adiponectin has a substantial effect on innate immunity, which is reviewed elsewhere [251]. Adiponectin protects against oxidative stress in vascular endothelial cells by stimulating NO synthesis in an AMPK-dependent manner and suppressing expression of the NOX2 subunit gp91phox [43,252]. As alluded to earlier in this review, another mechanism by which adiponectin ay suppress inflammation is mediated by the ceramidase activity of AdipoR1 or AdipoR2 thereby removing pro-inflammatory ceramides [28,44].

Given these actions, reduced adiponectin during obesity likely contributes to metainflammation, exacerbating insulin resistance. Furthermore, hyperinsulinaemia during insulin resistance may also contribute to metainflammation, with both hyperinsulinaemia and reduced adiponectin therefore increasing cardiovascular disease risk.

PHARMACOLOGICAL TARGETING OF NUTRIENT-REGULATED INFLAMMATION

Diet, physical activity and metainflammation

Caloric restriction (CR) is the only intervention known to reliably extend healthy lifespan in primates by delaying the onset of age-related conditions such as diabetes and cardiovascular disease [253]. However, long-term adherence to CR regimens is challenging, which has prompted the search for pharmacological caloric restriction mimetics. As described earlier, PUFA have been reported to have largely anti-inflammatory actions in adipose and vascular tissues [94,95]. Despite these beneficial actions of PUFA in cultured cells and rodent models, human dietary interventions have, however, provided inconsistent results [100].Furthermore, increased physical activity improves insulin sensitivity whilst reducing cardiovascular

disease mortality and morbidity [254]. Many of the benefits of exercise on cardiovascular health are due to the introduction of pulsatile shear stress to the vascular endothelium [255], which includes enhancing the expression of antioxidant proteins and reducing levels of pro-inflammatory cytokines, including IL-6 and TNFα [256]. However, the effects of aerobic exercise training reported on markers of inflammation associated with T2DM are inconsistent [257].

Effects on metainflammation of existing hypoglycaemic and cardiovascular therapeutics

As evidence for a role for metainflammation in insulin resistance and cardiovascular disease has increased, there has been a parallel increase in evidence supporting anti-inflammatory effects of hypoglycaemic and cardiovascular therapeutics. As mentioned before, several hypoglycaemic drugs used to treat T2DM including thiazolidinediones, metformin, SGLT2 inhibitors and incretin mimetics reduce pro-inflammatory signalling [14]. When considering cardiovascular therapeutics, the reduction of LDL cholesterol levels by statins suppresses vascular inflammation [258]. Furthermore, statins have multiple anti-inflammatory effects that are independent of their lipid-lowering action, reducing chemokine secretion and ICAM-1 levels in human monocytes [259], TNFα and interferon-γ production in T-lymphocytes and inhibiting Th-1 polarisation (reviewed in [260]). Furthermore, addition of statins to endothelial cells inhibits TNFα signalling [261], whereas statins can reduce vascular oxidative stress and inflammatory markers to reduce monocyte recruitment and adhesion [262,263].

Hypertension is the most important modifiable risk factor for heart failure, stroke and chronic kidney disease. The renin angiotensin aldosterone system, mainly through angiotensin II (Ang II), plays a key role in reducing NO production and bioavailability, thereby stimulating production of free radicals and pro-inflammatory molecules [264]. Angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) inhibit the production and action of Ang II respectively, and have both been shown to limit vascular inflammation. In addition to counteracting Ang II-mediated effects, ACE inhibitors also inhibit degradation of bradykinin, thereby enhancing NO release and improving endothelial-dependent vasodilatation [265], whereas ARBs, through selective AT1 receptor blockade, permit Ang II binding to AT2 receptors to stimulate NO production [266,267]. There is significant evidence, therefore, that

many hypoglycaemic and cardiovascular therapeutics have anti-inflammatory actions that may contribute to their efficacy.

Targeting nutrient sensors to limit metainflammation

As discussed earlier, three intracellular pathways that sense nutrient sufficiency and deprivation are the PKC, mTOR and AMPK pathways, all of which have been proposed to regulate chronic inflammation in the development of age-related cardiovascular diseases.

Due to the role of PKC in mediating the effects of overnutrition/hyperglycaemia on inflammation and insulin sensitivity, clinical trials have been conducted with small molecule inhibitors of PKC isoforms for diabetic nephropathy and retinopathy, yet no PKC-targeting drugs have been approved for use in those conditions [268].

The mTOR inhibitor rapamycin and its analogues, termed “rapalogs”, are used for suppression of organ rejection after kidney transplantation, inhibition of vascular re-stenosis and treatment of renal cell carcinoma [269]. Importantly, studies in diverse model organisms strongly implicate mTOR in the ageing process, with mTORC1 inhibition increasing lifespan [270,271]. While the mechanisms responsible are unclear, one consequence of mTOR inhibition is stimulation of autophagy, which helps clear cells of damaged proteins and mitochondria that accumulate in age-related diseases [271]. While mTORC1 is the immediate direct target of rapamycin, long-term rapamycin treatment also results in mTORC2 inhibition, thereby impairing insulin signalling [272]. It is worth noting that long-term mTOR inhibitor treatment by either intermittent dosing with rapamycin or using rapalogs (everolimus, temsirolimus) with different pharmacokinetic properties could be one way to achieve beneficial mTORC1-mediated effects on longevity with minimal mTORC2-mediated adverse effects of immunosuppression and glucose intolerance [273]. Thus, mTORC1 inhibitors that can selectively target those processes involved in longevity while minimising side effects hold even greater promise.

The hypoglycaemic drugs metformin and canaglaflozin are currently used for management of T2DM and also activate AMPK, although this is not their principal mechanism of action (Figure 5) [161]. Metformin is widely used, relatively safe, inexpensive and might, therefore, be beneficial for long-term treatment regimens. Common side effects, such as hypoglycaemia and gastrointestinal intolerance which can occur in up to 30% of patients, are relatively mild. Several observational studies

have reported that treatment with metformin limits cardiovascular morbidity and mortality independent from its glucose-lowering action in patients with T2DM [274-277]. In support of a role for metformin in limiting chronic vascular inflammation responsible for cardiovascular disease, we and others have shown that metformin can suppress JAK-STAT and NF-κB pro-inflammatory signalling via multiple AMPK-dependent mechanisms [27,31]. However, clinical studies have shown minimal effects on surrogate markers of cardiovascular disease in non-diabetic patients with high cardiovascular risk either taking statins [278] or following cardiac surgery [279]. There is on-going development of more specific direct AMPK activators includinga proof-of-concept phase IIa clinical trial in people with T2DM where an AMPK activator was demonstrated to reduce fasting plasma glucose levels and insulin resistance [280] and NCT04321343, an ongoing trial of a different direct AMPK activator in nonalcoholic hepatic steatosis. It is not unreasonable, therefore, that selective activation of AMPK complexes present in vascular cells represents a feasible approach to reduce the chronic inflammation responsible for cardiovascular disease.

CONCLUSIONS

The mechanisms described in this review highlight the myriad ways by which nutrients regulate and contribute to the metainflammation that occurs during atherogenesis and in obese adipose tissue. There is, however, much still to understand concerning the actions of specific nutrients, particularly how different saturated and unsaturated fatty acids influence inflammation signalling pathways. In this regard more information concerning the roles of FFAR1 and FFAR4 in inflammation and lipid signalling will be highly instructive. This review has not considered the roles of micronutrients including vitamins and metabolites generated by microbiota in the regulation of adipose and vascular tissue inflammation, for which the reader is directed to recent reviews [281,282].Furthermore, although many actions of nutrients have been described in vascular and adipose tissue, most research in leukocytes has focussed on macrophage behaviour, despite it becoming clear that multiple leukocyte types are involved in regulating inflammation within these tissues. In addition, adipose tissue may have a more direct impact on vascular function as more studies indicate a critical role for PVAT in the regulation of vascular health.It should also be noted that most mechanistic studies have been undertaken

in cultured cells, isolated animal tissues or animal models and there is a great need for further understanding of how the mechanisms and pathways are altered in human pathophysiology. Despite this, as highlighted in this review, regulation of AMPK and mTORC1 permit a coordinated response to circulating nutrients that includes regulation of inflammation signalling. In contrast, overnutrition activates PKC that has multiple actions to exacerbate inflammation signalling. Targeting these key kinases may therefore have therapeutic benefits by suppressing excessive pro-inflammatory signalling in obesity-related complications including atherosclerosis.

FUNDING

The laboratories of TMP and IPS have been supported recently by Ph.D Studentships (BDA09/0003948 and BDA11/0004403) and an equipment grant (BDA11/0004309) from Diabetes UK and Project Grants (PG/12/1/29276 and PG/13/82/30483)from the British Heart Foundation (BHF) TMP's laboratory is currently funded by a project grant from the Hull and East Riding Cardiac Trust Fund.

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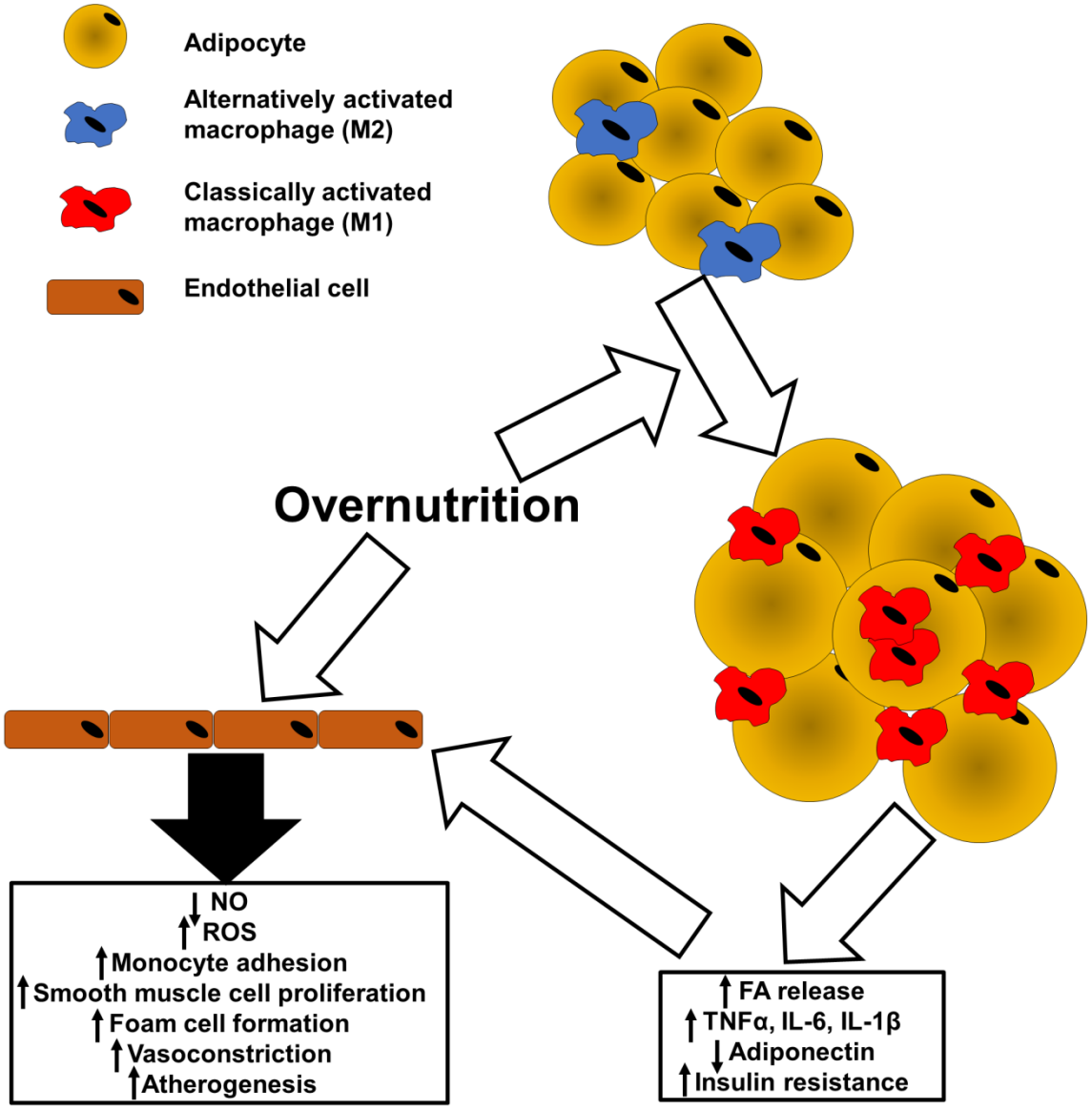

Figure 1: Adipose tissue inflammation, endothelial dysfunction and atherogenesis

Pathological adipose tissue expansion due to sustained overnutrition results in adipocyte hypertrophy, fibrosis and death, with increased numbers of adipose tissue macrophages that are polarised towards a pro-inflammatory (M1) phenotype. This alters the secretory profile of adipose tissue, increasing FA release and pro-inflammatory cytokine secretion whilst reducing adiponectin secretion, further exacerbating insulin resistance. Increased pro-inflammatory cytokine and FA levels can accelerate endothelial dysfunction, whereby inflammation within the vascular wall contributes to atherogenesis with reduced NO bioavailability, increased

monocyte recruitment and foam cell formation. Overnutrition can also directly influence endothelial dysfunction by altered nutrient metabolism.

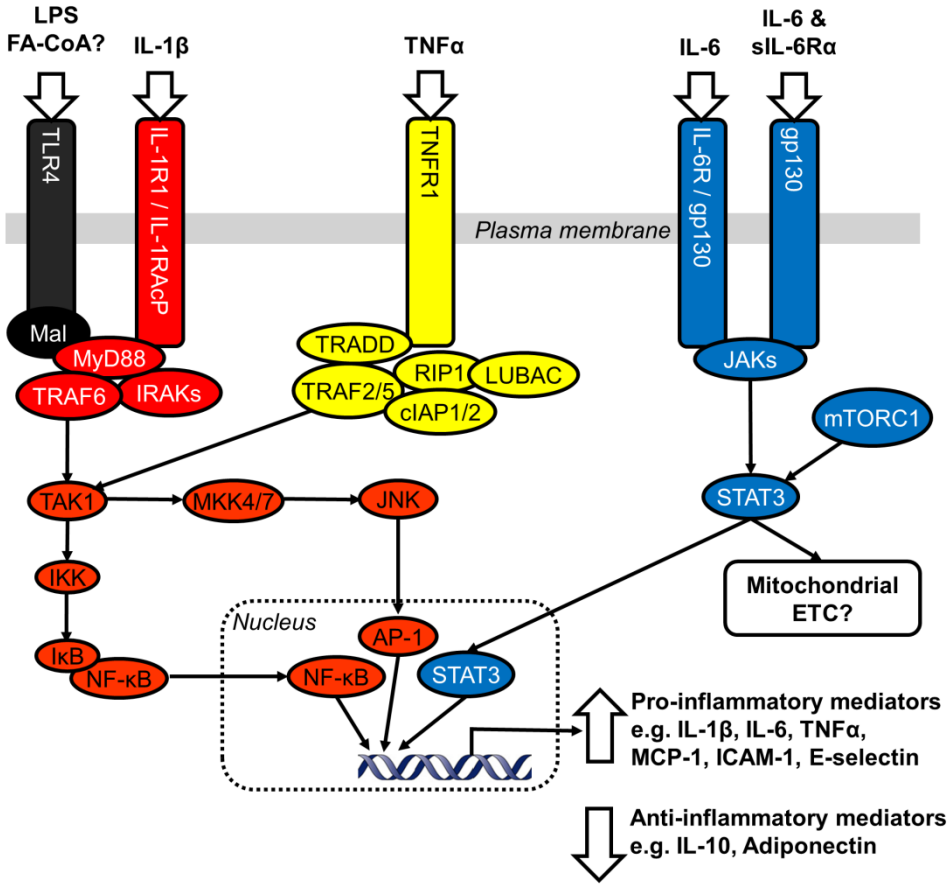

Figure 2: Pro-inflammatory cytokine signalling

IL-1 activates a dimeric IL-1R/IL-1RAcP complex at the plasma membrane, resulting in the recruitment of adaptor protein MyD88. Similarly, activation of dimeric TLR4 receptors by LPS recruits MyD88 to the membrane via the adaptor Mal. This leads to sequential activation of IRAK4, IRAK1 or IRAK2, TRAF6, TAK1 and IKK. IKK phosphorylates IκBα, which subsequently is degraded, leading to nuclear translocation of heterodimeric p65/p50 NF-κB. TAK1 also phosphorylates MKK4/7, leading to activation of JNK which phosphorylates the transcription factor AP-1. Activation of trimeric TNFR1 complexes by TNFα results in recruitment of a multi-protein complex containing adaptor protein TRADD, RIP1, TRAF2, TRAF5, cIAP1, cIAP2 and LUBAC. This complex then recruits and activates TAK1 and IKK.

IL-6 complexed with either a membrane-bound or soluble IL-6 receptor (sIL-6Rα) binds a dimeric gp130 complex at the plasma membrane. This triggers activation of JAKs which phosphorylate specific Tyr residues on the cytoplasmic domain of gp130. These act as recruitment sites for STAT transcription factors (mainly STAT3). Recruited STAT3 proteins are phosphorylated on Tyr705 by gp130-bound JAK and homodimerise. STAT3 is also phosphorylated on Ser727 by several protein kinases including mTORC1. Upon translocation to the nucleus, STAT3 dimers bind specific promoters and recruit transcriptional co-activators to initiate gene transcription. Mitochondrial localisation of phosphorylated STAT3, where it regulates the electron transport chain (ETC) to limit generation of ROS, has also been reported in adipocytes and other cells.

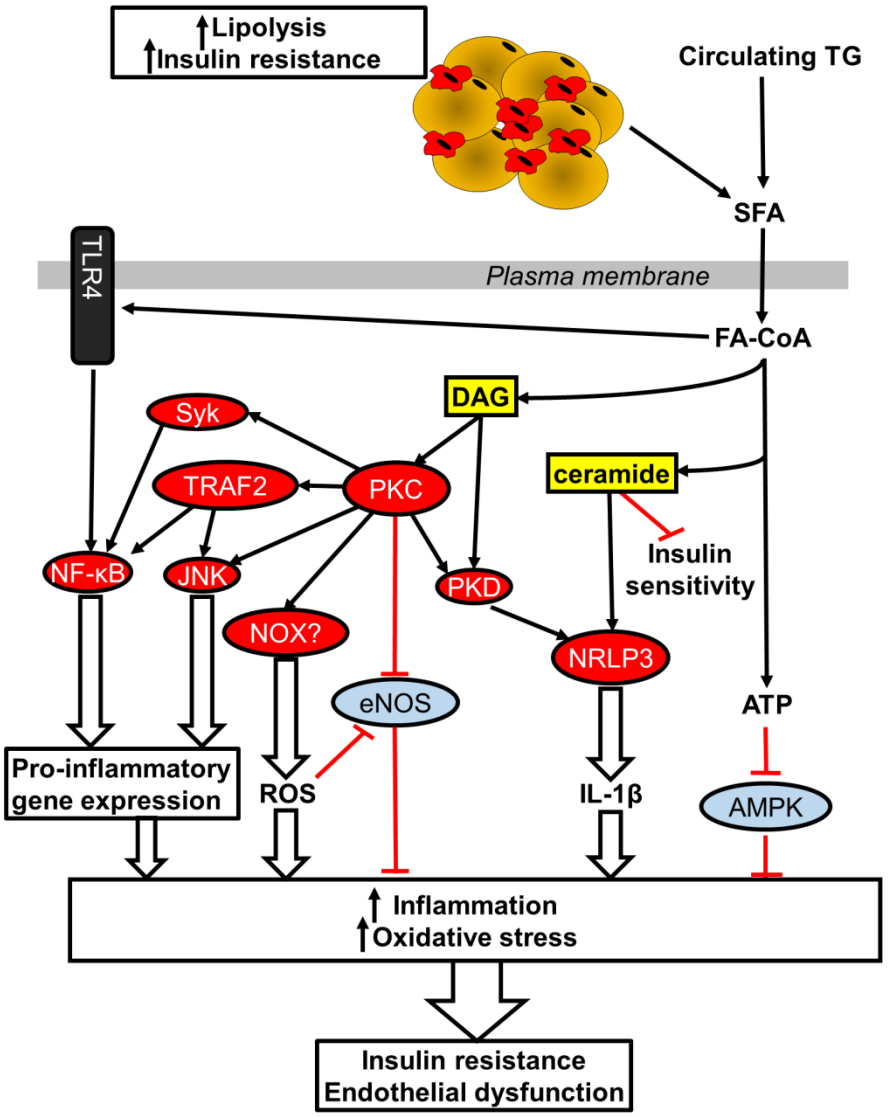

Figure 3: Regulation of inflammatory signalling by saturated fatty acids

Insulin resistant adipose tissue releases increased SFAs due to increased lipolysis. SFAs stimulate TLR4, most likely via increased FA-CoA concentrations. DAG synthesis is also increased in response to SFAs, leading to activation of PKC, which promotes NF-κB and JNK activation by cytokines and TLR4 activation. Activation of PKC also stimulates ROS synthesis and inhibits production of NO, thereby suppressing the anti-inflammatory actions of NO. PKD activation subsequent to PKC has been reported to stimulate NRLP3 inflammasome activation. Ceramide concentrations are increased by high SFA concentrations, impairing insulin

sensitivity via reduced Akt activation. PKC also impairs insulin sensitivity by phosphorylating insulin receptor substrate 1, not shown on the figure.

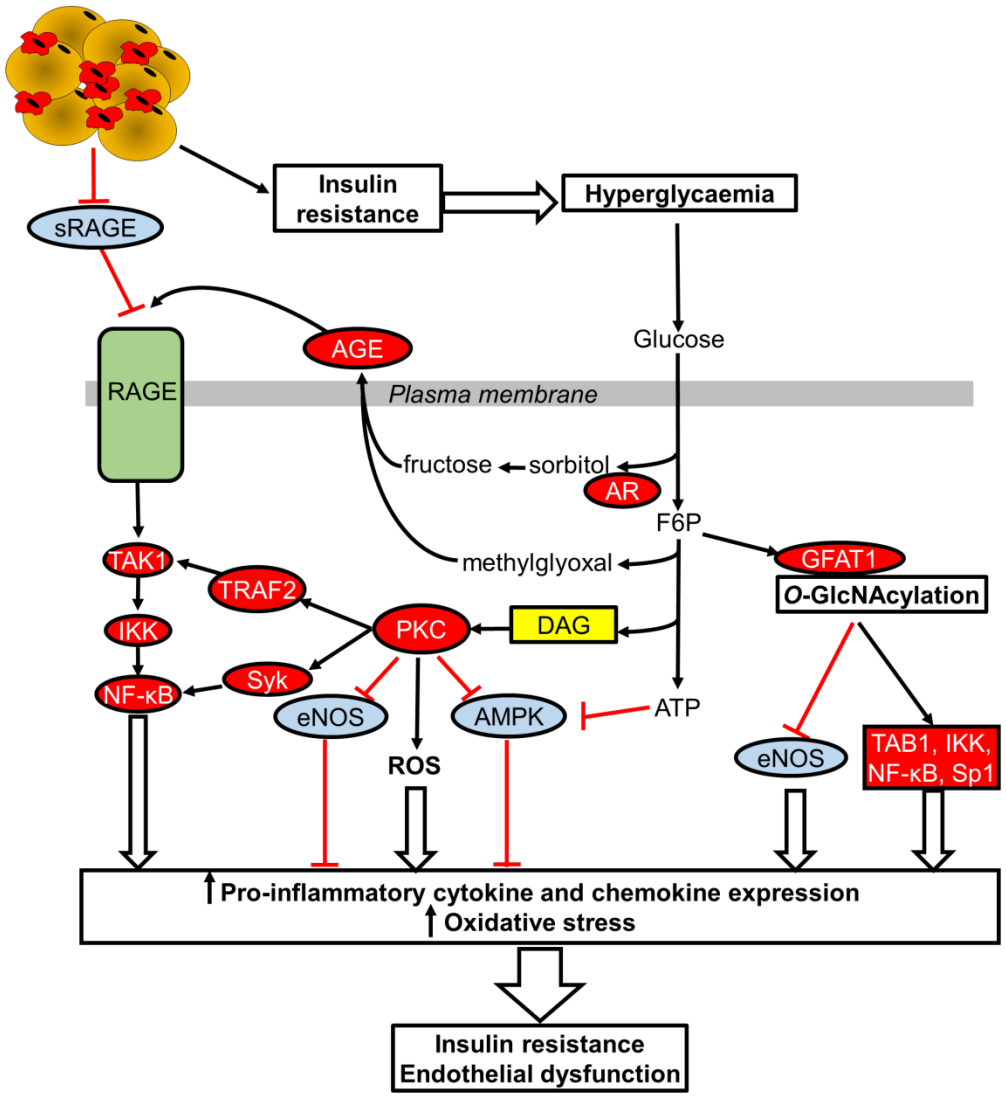

Figure 4: Regulation of inflammatory signalling by glucose metabolism

Hyperglycaemia due to insulin resistance leads to increased flux through the polyol pathway due to aldose reductase (AR), and synthesis of methylglyoxal from triose phosphate intermediates. These lead to the synthesis of AGE which signal via RAGE to stimulate NF-κB activation and other pro-inflammatory signalling pathways. Increased activity of GFAT1 leads to*O*-GlcNAcylation and inhibition of eNOS and has been reported to increase *O*-GlcNAcylation of TAB1, IKKβ and NF-κB. Hyperglycaemia also leads to increased levels of DAG, activating PKC, leading to stimulation of NF-κB. The mechanism for this may involve PKC-mediated activation of Syk and TRAF2. Activation of PKC also stimulates ROS synthesis, whilst inhibiting

production of NO by eNOS. Furthermore, PKC may directly phosphorylate and inhibit AMPK, thereby suppressing the anti-inflammatory actions of AMPK and NO.

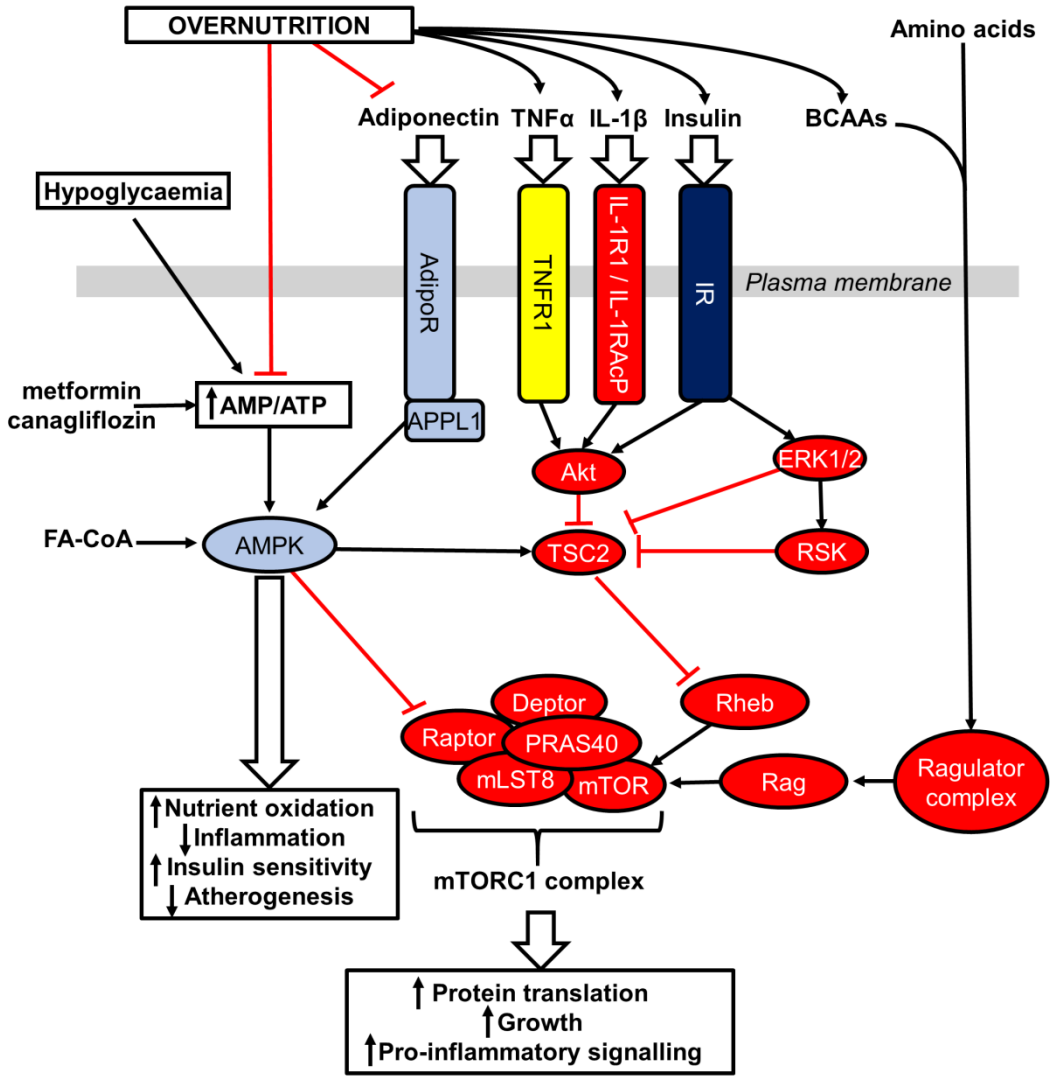

Figure 5: Regulation of AMPK and mTORC1 by nutrients and growth factor signalling

AMPK is activated by increases in the AMP/ATP ratio, which occurs in response to hypoglycaemia and anti-diabetic medications including metformin and canagliflozin. In contrast overnutrition suppresses AMPK by as yet uncertain mechanisms and more recently FA-CoA has been demonstrated to allosterically activate AMPK. The anti-inflammatory adipocytokine adiponectin also activates AMPK via APPL1. TNFα and IL-1β receptor activation stimulates Akt, which phosphorylates and inhibits tuberous sclerosis complex-2 (TSC2), which acts in a complex to inactivate Rheb (GTPase Ras homologue enriched in brain). Upon inhibition of TSC1/2, GTP-bound

Rheb levels increase to trigger activation of mechanistic target of rapamycin complex 1 (mTORC1) at the lysosome. Key proteins within the mTORC1 complex are indicated. Growth factors including insulin also stimulate Akt and ERK1/2. ERK1/2 and its substrate RSK (p90 ribosomal S6 kinase) can also can phosphorylate and inhibit TSC2. Conversely, AMPK phosphorylates Raptor and TSC2, leading to inhibition of mTORC1. Amino acids stimulate the Ragulator complex, which promotes accumulation of GTP-bound Rags. The active Rags bind mTORC1 and recruits it to the lysosome.

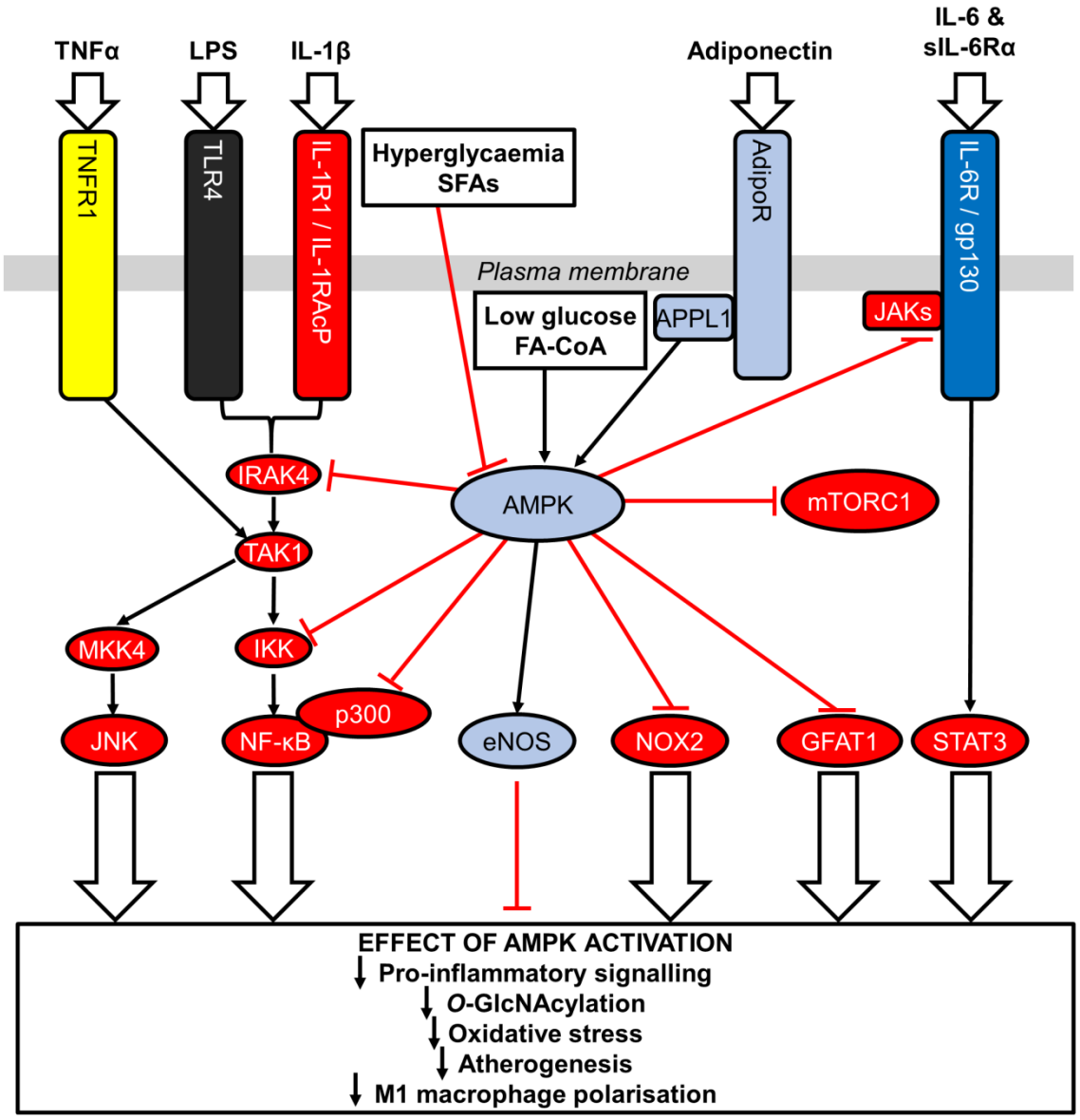

Figure 6: Anti-inflammatory actions of AMPK

Active AMPK inhibits many pro-inflammatory signalling pathways, including phosphorylation and inhibition of JAK in response to IL-6, and NF-κB activation in response to TNFα, IL-1β and TLR4 stimulation. AMPK activation inhibits IRAK4 autophosphorylation and has been proposed to inhibit NF-κB activation by inhibitory phosphorylation of IKK or p300. AMPK has also been demonstrated to phosphorylate and inactivate GFAT1, thereby suppressing *O*-GlcNAcylation. Phosphorylation of eNOS by AMPK leads to NO synthesis, whereas AMPK also inhibits NOX2-mediated synthesis of ROS.