

**The Role of Metabolite-sensing G Protein-Coupled Receptors in **inflammation** and **metabolic** disease**

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## **Abstract**

Great attention has been placed on the link between metabolism and immune function. **giving rise to the term “immunometabolism”**. It is widely accepted that inflammation and oxidative stress are key processes that underlie metabolic complications during obesity, diabetes and atherosclerosis. Therefore, identifying the mechanisms and mediators that are involved in the regulation of both inflammation and metabolic homeostasis is of high scientific and therapeutic interest. G protein-coupled receptors (GPCRs) that signal in response to metabolites have emerged as attractive therapeutic targets in inflammatory disease. In this review, we discuss recent findings about the physiological role of the main metabolite-sensing GPCRs, their implication in immunometabolic disorders, their principal endogenous and synthetic ligands and their potential as drug targets in inflammation and metabolic disease.

**Keywords:** **immunometabolism, diabetes,** inflammation, oxidative stress, G protein-coupled receptors.

The traditional view that the role of the immune system is limited to combating pathogens has been challenged over the past decade. It is now recognised that the immune system plays many different roles in regulating endocrine, neural and behavioural responses. Increasing evidence supporting a link between metabolism and immune function has led to the emergence of the term ‘immunometabolism’. This concept first appears in PubMed in a foreword to the February 2011 issue of Nature Reviews Immunology by Diane Mathis and Steven Shoelson (99). The authors coined the term to highlight regulatory interactions between innate and adaptive immunity and metabolism. Until around 5 years ago few immunologists appreciated the role metabolism could play in effector cell function and few scientists working in the area of metabolism or systems physiology had fully appreciated the way immune cells impact on metabolic flux and the function of metabolic tissues including white adipose tissue (WAT) and the liver. The term immunometabolism has been given further prominence as we rediscover the important role of metabolic reprogramming in inflammation (69) and cancer biology (126). The value of immunometabolism as a field of scientific study in its own right will be judged ultimately by its success in delivering new insights into the biology of cells of the innate and adaptive immune system, new insights into disease mechanisms and the identification of new targets for therapeutic intervention in metabolic disease.

In this brief review, we will confine our discussion to one specific aspect of immunometabolism, namely the link between **innate immune response and metabolic disorders focusing on** the G protein-coupled receptors (GPCRs) that have cell or microbial metabolites as agonists. Such receptors are of especial interest to physiologists, pharmacologists and pathologists for two reasons. First, GPCRs that sense changes in cellular metabolites within the physiological range of concentrations are well placed to transduce the effects of changes in cell metabolism on immune cell function. Secondly, new insights into GPCR biology such as biased agonism (170) and structural studies of GPCRs (41) offer new avenues for more effective drug discovery and drug design (both agonists and antagonists). Here we highlight mammalian GPCRs that have been shown to signal in response to cell metabolites and we critically review the role these metabolite-sensing GPCRs play in immunity, oxidative stress and disease pathology.

## Metabolic diseases

It is known that metabolic diseases have become a worldwide epidemic of the 21st century. This is especially true of obesity and diabetes mellitus, for both of which global incidence has reached dramatic proportions. This alarming rise in metabolic disorders has been attributed to changes in lifestyle; a predominance of sedentary habits and an excessive consumption of food rich in fats and refined carbohydrates (117,125). The World Health Organization estimates that 600 million people worldwide are clinically obese (122). Obesity is defined as an excessive expansion of adipose tissue and has been recognized as a driving force in the development and progression of comorbidities, such as type 2 diabetes (T2D), insulin resistance, hypertension, dyslipidaemia, atherosclerosis, and fatty liver disease, among others (44,51,116). Collectively, these non-communicable pathologies constitute a major threat to global human health.

Obesity is a great contributor to inflammation and oxidative stress (129). The excess high-fat and carbohydrate diets trigger an increase in oxidative stress as a consequence of a higher number of lipid peroxidation products and a decreased antioxidant activity. Abundant evidence indicates that this oxidative stress and the associated inflammation contribute to the onset and progression of metabolic pathologies including **T2D** and insulin resistance (24,112,128,172). Tissue inflammation, the synthesis of reactive oxygen species (ROS) and intracellular signalling pathways are strictly regulated to maintain homeostasis and survival. However, in the pathological environment of obesity, an imbalance in redox regulation, impaired inflammatory signalling and dysfunction in insulin secretion and signalling occur with potentially severe consequences (112). It is hoped that a better understanding of the mechanisms that underlie the progression of metabolic disorders may allow us to develop new therapeutic strategies to decrease the prevalence of obesity, diabetes and therefore cardiovascular disease worldwide.

### *Oxidative stress in metabolic diseases*

Elevated oxidative stress is a key mechanistic link between obesity and its associated metabolic complications (180). Oxidative stress emerges as an imbalance between ROS generation and the activity of the antioxidative systems of cells and tissues. ROS are low-molecular-weight chemically unstable compounds that derive from molecular oxygen and include superoxide anion ( $O_2^-$ ), hydroxyl radical ( $\bullet OH$ ), hydrogen peroxide ( $H_2O_2$ ), and peroxynitrite ( $ONOO^-$ ) (65,66,187). Although ROS signalling is essential in cell protection

and survival, an overproduction of oxidative free radicals can result in the modification of the structure and function of biological macromolecules, including DNA damage, lipid peroxidation and membrane protein damage, leading to cellular dysfunction (65). The results of ROS overproduction can include impaired energy metabolism, aberrant cell signalling, cell cycle control and transport mechanism, immune activation and inflammation (58,66).

The major cellular sites of ROS generation are the mitochondrial electron transport chain, membrane-bound nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase (NOX) isoforms (1-5), dual oxidases (Duox 1 and 2), endoplasmic reticulum, and peroxisomes (112). As one the major sources of ROS in the cell, the mitochondria express the electron transport chain complexes which transfer electrons from NADH and succinate, along a controlled pathway, to the oxygen molecule ( $O_2$ ), which is then reduced to  $H_2O_2$ . When this mechanism is altered, the  $O_2$  undergoes one- or two-electron reduction to form  $O_2^-$  or  $H_2O_2$ , respectively (75).

Phagocytic cells importantly contribute to generate oxidative stress since they produce large amounts of ROS via NADPH oxidase to kill bacteria during the called *respiratory burst* (127). Furthermore, T cells are often present in close proximity to phagocytic cells and they can also trigger respiratory burst by direct contact with phagocytes or secreted cytokines (184).

ROS levels are regulated by antioxidant enzymes and ROS degradation to keep a non-toxic balance. Antioxidant defences include superoxide dismutase (SOD), catalase or glutathione peroxidase (GSHPx) (65,112).

Oxidative stress is increased in obese individuals, and reduced after weight loss (67,68). Patients with diabetes mellitus have high levels of ROS as well as serum peroxidized fat and oxidized low-density lipoproteins (LDL). In addition, increased ROS levels have also been observed in animal models of diabetes (25,65). High glucose concentrations promote an increase in the production of ROS in several cell types, including pancreatic  $\beta$ -cells. When intracellular glucose concentrations exceed the glycolytic capacity of such cells, excess glucose is shunted to enolization pathways, resulting in production of superoxide. Moreover, pancreatic  $\beta$ -cells decrease their expression of antioxidant enzymes, becoming extremely sensitive to oxidative stress (7). In addition to high glucose levels, fatty acids also constitute an important factor in ROS production in pancreatic cells. They control mitochondrial complexes and electron transport, activate NAD(P)H oxidase, induce uncoupling proteins,

interact with the renin-angiotensin system or modulate the antioxidant enzymes. The amphiphilic nature of fatty acids facilitates their incorporation into mitochondrial membranes which alters the membrane fluidity and favours electron leak (40).

### *Inflammation in metabolic diseases*

Chronic inflammation plays a key role in the progression of diabetes via abnormal cytokine production, increased recruitment, impaired proliferation, differentiation and altered migration of leukocytes; therefore, a network of inflammatory signalling pathways is globally activated (6,29,172). The first clear evidence that linked obesity, diabetes and chronic inflammation was the finding that the pro-inflammatory cytokine tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) was overexpressed in the adipose tissue of obese mice (52). Subsequently, numerous studies revealed that the chronic low-grade systemic inflammation associated with obesity is enhanced when immune-inflammatory cells such as macrophages and T cells infiltrate adipose tissue (22,60). M1, pro-inflammatory, macrophages have been shown to secrete high amounts of inflammatory mediators within the adipose tissue including chemokines (CCL2, CXCL5) and cytokines (TNF- $\alpha$ , interleukin (IL)-6, IL-18), which are then released to the bloodstream thereby contributing to systemic inflammation status (123). In peripheral tissues including liver, skeletal muscle and in adipose tissue itself, inflammatory mediators and inflammation-dependent ROS interact with the insulin receptor (IR) and its associated downstream pathways, leading to a dysregulation in insulin response mechanisms (167).

In all these processes that cause tissue damage and metabolic dysregulation, metabolite sensing GPCRs emerge as key mediators that can either promote or oppose inflammation, oxidative stress and impaired metabolism progression- see Figure 1.

### **Metabolite-sensing GPCRs**

Many key mediators of inflammation and its resolution signal via GPCRs. These receptors constitute the largest family of membrane proteins in mammals and they participate in the regulation of major physiological functions in the organism. GPCRs have the ability to bind to a wide variety of ligands with high affinity and specificity (2,14,35).

The explosion in messenger RNA (mRNA) and genome sequencing in the late 20th century led to the identification of multiple open reading frames containing seven membrane

spanning  $\alpha$ -helices reminiscent of GPCRs of known function (174). These so called ‘orphan receptors’ posed a significant challenge to biologists and pharmacologists because without knowledge of their cognate ligands a full understanding of their role in physiology and pathology was impossible. Within the wide family of GPCRs, a growing number have now been identified as metabolite-sensing GPCRs; this group of deorphanized receptors has been reported to be activated by intermediates of energy metabolism including free fatty acids (FFAs), lactate, succinate and ketone bodies, among others. Owing to their presence in key cell types regulating both metabolic and immune systems, many of them have been shown to have a pivotal role in the progression of metabolic disorders such as diabetes, obesity and dyslipidaemia and in the associated inflammatory response (3,14,179). Therefore, there is intense research interest in this subfamily of metabolic receptors that are emerging as promising therapeutic targets in several immunometabolic disorders, for an excellent recent review see Husted A.S et al. (54).

FFAs have been revealed to be not only important nutrients from dietary fat, but also key signalling molecules that mediate critical biological functions (103). According to the carbon chain length of the FFAs that activate them, the most accepted classification of the metabolite GPCRs divides them into different groups: those activated by long chain fatty acids (LCFA, C12-C22) including GPR120 (FFAR4) and GPR40 (FFAR1); medium chain fatty acid MCFA receptors (C7-C12) highlighting GPR84; and short chain fatty acid (SCFA) receptors (C2-C6) including GPR41 (FFAR3) and GPR43 (FFAR2) (3,164). Blad et al. presented an extended version of metabolite GPCRs classification by adding two other groups to the original list; the group of citric acid cycle intermediate receptors where succinate receptor (GPR91) is highlighted, and secondly the receptors activated by hydroxycarboxylic acids, such as GPR109 (13)- see Table 1.

Once activated by a ligand, metabolite GPCRs can couple to different families of heterometric G proteins (Gs, Gi/Go, Gq/G11 and G12/G13)- see **Figure 2**- and/or  $\beta$ -arrestins, then regulating the activity of second-messenger producing enzymes (phospholipases, adenylyl cyclases) or ion channels, which in turn modulate the intracellular concentrations of second messengers (inositol triphosphate (IP3), diacylglycerol (DAG), cyclic AMP (cAMP), calcium, among others) and therefore the downstream signalling within the cell, including different kinase cascades such as ERK/MAPK, JNK, p38, or the Akt/PI3K route. These pathways control cell proliferation, differentiation, survival, migration and other essential biological functions (2,39,165).

In this review, we summarize the main functions, signalling and expression patterns of the principal metabolic-sensing GPCRs in physiology and disease, highlighting the key role they play in inflammation during metabolic complications.

## **1. Long-chain fatty acid receptors**

Major LCFAs present in plasma include palmitic acid (C16:0), stearic acid (C18:0) and several unsaturated fatty acids such as oleic acid (C18:1) or linoleic acid (C18:2). Furthermore, the group of polyunsaturated omega-3 fatty acids ( $\omega$ 3-PUFAs), mainly  $\alpha$ -linolenic acid (ALA, C18:3), eicosapentaenoic acid (EPA, C20:5), and docosahexaenoic acid (DHA, C22:6), although not synthesized by the organism are essential for multiple normal physiological processes. These molecules have been shown to exert anti-inflammatory, antioxidant and anti-diabetic properties, actions which are thought to be GPCR-mediated (104). The potential therapeutic effect of metabolite-sensing GPCRs has been investigated in several diseases including cancer, diabetes, rheumatoid arthritis and inflammatory bowel disease (30,32,91,160). In the last decade, GPR120 and GPR40 have been identified as the most relevant metabolic GPCRs activated by LCFAs (46) -see Figure 3. Recent studies have shown that the administration of selective agonists for GPR120 and GPR40 improve glucose metabolism and ameliorate systemic metabolic disorders (85,94,140).

### **1.1. GPR120 (FFAR4)**

GPR120, also called FFAR4, is a rhodopsin-like GPCR subfamily member that was orphanized in 2005 and it is primarily expressed in adipose tissue, intestine (enteroendocrine L cells in colon), lung and macrophages (48,87,104). Its expression is highly conserved across many species (34). This receptor senses specific LCFAs including  $\omega$ -3 fatty acids and its stimulation results in an elevation of intracellular calcium concentration and an activation of the ERK cascade, thus suggesting interactions with the G $\alpha$ q family of G proteins (48).

GPR120 activation has been described to display insulin-sensitizing effects, participate in glucose homeostasis and ameliorate chronic low-grade inflammation in vivo (121). Furthermore, its activation by  $\omega$ -3 FFAs has shown to be involved in glucagon-like peptide-1 (GLP-1) secretion in the intestine. GPR120 acts as a lipid sensor in adipose tissue to sense dietary fat and control energy balance (87,104). In mice, GPR120 expression has been



detected in pancreatic delta cells regulating somatostatin secretion from islets (152). In addition, its expression has been described in the immune system, mainly in innate immune cells (121).

GPR120-deficient mice have shown to develop obesity, glucose intolerance, and hepatic steatosis. In addition, the lack of GPR120 is associated with impaired glucose metabolism and heightened lipogenesis when compared to control mice both fed a high fat diet (HFD) containing 60% fat. Likewise, in humans a dysfunctional variant of GPR120 is associated with metabolic complications including obesity (55). The anti-inflammatory effects of GPR120 have been widely observed in the immune system mainly in macrophages, a cell type where the receptor is highly expressed. Macrophage-expressed GPR120 couples to  $\beta$ -arrestin2 which is followed by receptor internalization and inhibition of both the toll like receptor-4 (TLR4) and TNF- $\alpha$  pro-inflammatory signalling pathways (121). It is well established that chronic inflammation is a key factor in the pathogenesis of insulin resistance involving the migration of monocytes and macrophages into adipose tissue and liver where they amplify inflammation by activating pro-inflammatory pathways and cytokine secretion, eventually leading, through paracrine effects, to decreased insulin sensitivity in nearby insulin secreting cells (138,144). In line with this, the anti-inflammatory effect of GPR120 in macrophages seems to be linked to insulin sensitivity in diabetic conditions.

Conflicting reports on the role of GPR120 in mediating the actions of  $\omega$ -3 FFAs are emerging. On one hand, most studies agree that GPR120 per se has an important role in energy homeostasis and inflammation independent of the presence or absence of  $\omega$ -3 FFAs (55,104,121,186). However, the role GPR120 plays in mediating the beneficial effects of  $\omega$ -3 FFAs by inducing an anti-inflammatory response and regulating insulin metabolism is discussed by Pærregaard et al. (124). In their latest study, and contradicting most existing literature, they proposed that GPR120 is dispensable for the beneficial effects that  $\omega$ -3 FFAs display in obese mice. GPR120 knockout mice and their controls were fed a with a high dose of  $\omega$ -3 FFAs and both groups showed protection against HFD-induced obesity, steatosis, insulin resistance, and visceral adipose tissue inflammation, consistent with no role for GPR120 in the therapeutic effects of  $\omega$ -3 FFAs.

Questions remain about the role of GPR120 in immunometabolism. The beneficial effect that this receptor in maintaining metabolic homeostasis has been widely accepted, thus GPR120 is emerging as a potential therapeutic target to treat diabetes and inflammation. The question

that needs to be clarified is which would be the optimal GPR120-based therapy: the use of selective GPR120 agonists, a modified diet rich in  $\omega$ -3 FFA components, or a combination of both?

## **1.2. GPR40 (FFAR1)**

GPR40, also called FFAR1, was first identified in 1997 (137). It is physiologically activated by saturated and unsaturated medium and long-chain FAs (C8:0-C22:0) as well as by conjugated linoleic acids (CLAs). Its expression has been described to be mainly in pancreatic  $\beta$ -cells, but GPR40 is expressed in the central nervous system, in the taste buds, in **innate** immune cells and enteroendocrine cells (16,21,28,57,93). In enteroendocrine cells GPR40 has been reported to participate in the production of regulatory peptides GLP-1 and gastric inhibitory polypeptide (GIP), which are key regulators of glucose metabolism (28).

Since its deorphanization, GPR40 has been seen as an interesting therapeutic target for T2D treatment due to its implication in mediating fatty acid mediated insulin secretion (36,57). However, conflicting data have emerged regarding the role of this G-coupled protein receptor in metabolic homeostasis. Stenberg et al. reported that mice lacking GPR40 were protected against effects of HFD, including hyperglycemia, hyperinsulinemia and glucose intolerance. In addition, transgenic mice overexpressing GPR40 in  $\beta$ -cells displayed a diabetic phenotype (150). These results support GPR40 antagonists as a potential therapeutic approach to prevent and treat T2D. A GPR40 antagonist (DC260126) has been reported to protect pancreatic  $\beta$ -cells against endoplasmic reticulum stress, dysfunction and apoptosis (153,175). Moreover, exendin-4, a GLP-1 mimetic drug, was shown to counteract the proapoptotic effect of palmitate in  $\beta$ -cells by reducing GPR40 expression (111).

In contrast to these findings, several studies have reported the beneficial effect of GPR40 activation in diabetes, increasing insulin secretion and improving glucose tolerance in murine  $\beta$ -cells (77,106). Numerous drugs that act as specific GPR40 agonists are either in clinical (TAK-875, JTT-851, P11187 and LY2881835) or in preclinical (CNX-011-67, SAR1, DS-1558 and BMS-986118) development (26,59,82). However, most of these tool compounds have been investigated for their beneficial effect in GPR40 mediated-insulin secretion without any attention focused in their relationship with FFA mediated-lipotoxicity. Studies have shown that peroxisome proliferator activated receptor gamma (PPAR $\gamma$ ) agonists, which activate GPR40, induce resistance to lipotoxicity in  $\beta$ -cells (46). In agreement with this, the anti-lipotoxic effect of the PPAR $\gamma$  agonist pioglitazone in  $\beta$ -cells has shown to be weakened

after silencing the expression of GPR40 (176). Shen et al. further revealed that the role of GPR40 mediating the anti-oxidative stress effect of pioglitazone occurs through activation of the phospholipase C (PLC) $\gamma$ -ERK1/2-PPAR $\gamma$  signalling pathway (142). Activation of PLC by GPR40 activation leads to the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) into DAG and IP3. DAG and IP3 potentiate insulin secretion by activating protein kinase C (PKC) and triggering endoplasmic reticulum (ER) Ca<sup>2+</sup> release, respectively (76).

Interestingly, a potential role of GPR40 in reducing inflammation has been reported. Activation of GPR40 by an agonist (CNX-011-67) was reported to attenuate inflammatory signalling by improving calcium flux and cAMP levels in  $\beta$ -cells. This anti-inflammatory role of GPR40 involves a reduction in inflammatory signalling molecules such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF $\kappa$ B) and JNK, in inflammatory cytokine gene expression, and in cellular oxidative and ER stresses (168). It will be interesting to explore the anti-inflammatory effects of this GPR40 agonist in other cell types including immune cells including macrophages and polymorphonuclear (PMN) cells. Recent evidence suggests that GPR40 is expressed in bovine neutrophils, where it displays an important role on PMN activation (96). Through PLC-PKC signalling, GPR40 activation by oleic and linoleic acids triggers the release of metalloproteinase-9 (MMP-9) granules and ROS production in neutrophils. Therefore, depending on the ligand that binds the receptor, the cell type and the tissue-environmental conditions, different roles on inflammation can be attributed to GPR40.

The expression of GPR40 in the brain has been extensively described, where this receptor exerts interesting neuroprotective properties (107-109). Mice lacking GPR40 were shown to develop inflammation and insulin resistance induced by CLAs in the brain, thus providing evidence for a protective role of the receptor in neuronal inflammation (136). This neuroprotection may come from GPR40 dependent stimulation of insulin and GLP-1 secretion (28,139). Activation of GPR40 signalling at the spinal level also appears to be effective in reducing peripheral inflammation and nerve injury-induced pain, suggesting that GPR40 could be a promising therapeutic target for new analgesic drug development (64).

Taken together, the potential role of GPR40 in improving insulin resistance, maintaining glucose homeostasis and preventing inflammation in pancreatic  $\beta$ -cells makes a compelling case for GPR40 as a drug target in metabolic disease. Current clinical studies indicate that specific GPR40 activation in pancreatic  $\beta$ -cells achieves the initially therapeutic endpoint for

the treatment of T2D, and consequently the pharmaceutical industry has shown a clear interest in developing effective GPR40 agonists.

## **2. *Medium-chain fatty acid receptors***

### **2.1. *GPR84***

An open reading frame predicted to encode a protein with seven membrane spanning  $\alpha$ -helices was independently identified by two groups in 2001 and termed GPR84 (174,181). Wang et al. showed that CHO cells transfected with a human GPR84 expression vector along with an aequorin reporter and G $\alpha$ 16, Gqi9 and Gqs5 expression vectors exhibited concentration dependent luminescent signalling with the MCFAs capric –see compound 1 in Figure 4 - and undecanoic acid (C11:0). Similar results were obtained with CHO cells transfected with a murine GPR84 expression vector (85% amino acid homology with human GPR84). Using stably transfected GPR84-CHO cells the authors demonstrated EC<sub>50</sub> values for capric acid in cAMP inhibition assays of 4  $\mu$ M, undecanoic acid of 8  $\mu$ M and dodecanoic (lauric) acid (C12:0) of 9  $\mu$ M. Importantly the authors found no evidence of GPR84 signalling in transfected cell systems in response to short chain fatty acid ligands of GPR43 or long chain fatty acid agonists of the GPR120 receptor. Wang et al. further demonstrated high-level expression of human GPR84 mRNA in human peripheral blood leukocytes, specifically neutrophils and lipopolysaccharide (LPS) stimulated monocytes. Structural homology searches show only limited amino acid sequence homology of GPR84 with free fatty acid receptors FFAR1-4. Rather, GPR84's closest homologues seem to be monoamine receptors (171). Finally, a potential role for GPR84 in inflammation was suggested by the authors' observation that LPS-stimulated RAW264.7 cells treated with a GPR84 agonist diindolylmethane 2 (DIM) – also active at the cannabinoid receptor CB2 (178) or the MCFAs capric, undecanoic and lauric acid induced IL-12 p40 mRNA and that this effect of GPR84 ligands was not observed without prior LPS treatment. The authors interpreted this to mean that GPR84 agonists acting via the macrophage GPR84 receptor amplified macrophage inflammatory responses. Hence GPR84 is considered a pro-inflammatory GPCR and physiological GPR84 agonists are considered pro-inflammatory.

Suzuki et al. reported that a novel GPR84-specific tool compound 6-n-octylaminouracil 4 (6-OAU) is more potent than hydroxylated or native MCFAs (EC<sub>50</sub> values of ~500nM for 6-

OAU versus  $\sim 4\mu\text{M}$  for capric acid) (154). The novel GPR84 surrogate agonist 6-OAU was shown to enhance IL-8 secretion from LPS-stimulated human neutrophils. Further small molecule screening programmes identified more agonists active at the human GPR84 receptor expressed in transfected cell systems. Zhang et al. screened a HEK/G $\alpha$ 16/hGPR84 cell line with a 160,000-compound library in agonist and antagonist mode and identified compound 5 –see Figure 4- as a GPR84 specific agonist with a 5-fold increased potency compared to 6-OAU (185). The same group subsequently performed a structure activity study based on the compound 5 and identified the 6 – see Figure 4- as the most potent GPR84 agonist so far described  $\text{EC}_{50}$  352pM versus  $\text{EC}_{50}$  438nM for 6-OAU in the same transfected cell line calcium flux assay (90).

Consideration of the relative potencies of ‘surrogate’ and ‘physiological’ agonists of the GPR84 receptor raises an important question - why is signalling at this GPCR by physiological MCFAs so weak compared to that obtained with tool compounds? One potential explanation is that results obtained from transfected cell line assays are poorly representative of GPR84 signalling in primary cells in vitro or in vivo. An alternative explanation is that physiological agonists other than MCFAs may exist in vivo, perhaps in inflamed adipose tissue or atherosclerotic lesions, or that GPR84 in vivo is part of a GPCR oligomeric signalling complex which responds to MCFAs in vivo differently to the way GPR84 alone does when transfected into cell lines in vitro. Additionally consideration must be given to the possibility that MCFA concentrations could differ considerably between different anatomical sites. For instance enteroendocrine cells in the gut could well be exposed to higher concentrations and a wider range of MCFA metabolites.

In 2005, Venkataraman and Kuo reported the generation of GPR84 knockout mice (166). Mice lacking the GPR84 gene were healthy and fertile and showed no defect in T lymphocyte or B lymphocyte proliferation to a range of mitogens. However, CD3<sup>+</sup> T cells of *Gpr84*<sup>-/-</sup> mice showed increased IL-4 gene transcription and IL-4 production when stimulated with anti-CD3 antibodies. Differentiation of *Gpr84*<sup>-/-</sup> T cells in vitro under Th2 conditions resulted in decreased secretion of the Th2 cytokines IL-4, IL-5 and IL-13 but no change in serum levels of these cytokines was seen in vivo after ovalbumin immunization. Subsequent studies identified differences between *Gpr84*<sup>-/-</sup> and wild-type mice in a partial sciatic nerve ligation model of neuropathic pain and accelerated cognitive decline and decreased numbers of microglia in a mouse model of Alzheimer’s disease (113). No obvious difference was seen in the pathogenesis of experimental autoimmune encephalomyelitis (EAE) or mouse

behaviour post endotoxin administration in Gpr84<sup>-/-</sup> compared to wild-type mice (8). In looking for potential mechanistic explanations of the effect of Gpr84 gene deletion on pain perception Nicol et al. performed a differential gene expression analysis of peritoneal macrophages treated with LPS for 3 hours and identified multiple changes in gene expression consistent with attenuated pro-inflammatory signalling in Gpr84<sup>-/-</sup> macrophages (113). There are currently no published reports linking genetic variation in the human GPR84 gene locus with human disease.

### 3. *Short-chain fatty acid receptors*

SCFAs mainly acetate (C2:0), propionate (C3:0) and butyrate (C4:0), are produced in the colon by gut microbial fermentation of dietary fibre and they are emerging as important regulators of inflammatory responses (155). Their biological functions in energy metabolism homeostasis and immune response are mainly linked with the activation of the G-coupled metabolic receptors GPR41 and GPR43 (4). Interestingly, the combination of SCFAs and their associated receptors has recently been suggested as the potential link between diet, gut microbiota and inflammatory response (97). Indeed, epidemiological studies have connected the increased incidence of inflammatory disorders and intestinal cancers to the consumption of diets poor in fibre and rich in refined carbohydrates (147).

GPR41 and GPR43 are mainly expressed in colon epithelial cells close to the major site of SCFA production, and also in adipose tissue, pancreatic islets, and immune cells (4,17,114). Their activation has been reported to be involved in different chronic inflammatory and metabolic diseases such as rheumatoid arthritis, inflammatory bowel disease (IBD), asthma, colon cancer, diabetes and obesity, but so far whether their role is beneficial or detrimental is controversial between different studies (13,37,70,98,100,161,163).

Both SCFA receptors respond to the same endogenous ligands, but GPR41 and GPR43 couple to different downstream signalling pathways (72). After activation by SCFAs, GPR41 selectively couples with G<sub>ai</sub>, inhibiting adenylyl cyclase, thereby decreasing intracellular cAMP concentration. In contrast GPR43 binds both G<sub>ai</sub> and G<sub>aq</sub>, which not only decreases cAMP but also increases cytoplasmic calcium levels (17,80). Furthermore, a recent study revealed that GPR43 can regulate NF $\kappa$ B pathway by coupling with  $\beta$ -arrestin 2, but there is no report linking GPR41 to  $\beta$ -arrestins (81).

### **3.1. GPR41 (FFAR3)**

There is little information about the role of GPR41 in inflammation and metabolism. It is known that GPR41 is broadly expressed in the pancreas, intestine, monocytes, T and B cells, and spleen. Although its expression was first described in human and rodent adipose tissue, current controversy exists about GPR41 expression in this tissue, because several groups have tried to detect it without success (17,80,177) . However, others have detected GPR41 expression, reporting that SCFA-induced GPR41 activity stimulates the production of the hormone leptin in mouse white adipose tissue cells (177,182). GPR41 is also expressed in enteroendocrine cells where it has been implicated in SCFA-mediated GLP-1 and PYY hormone secretion, thus participating in metabolic homeostasis (133,158). Few studies have reported a role for GPR41 role in inflammation. In one of them, Kim et al. showed that mice lacking GPR41 presented reduced colitis, whereas in a later study Trompette and co-workers reported exacerbated asthma in GPR41 deficient mice (70,161).

There is current absence of publications reporting a significant effect of GPR41 modulation in disease models, compared to the other SCFA receptor GPR43. Regarding its expression in T and B cells, further studies would be interesting to elucidate if this receptor participates in the regulation of the adaptive immune response.

### **3.2. GPR43 (FFAR2)**

Deorphanization of this receptor took place simultaneously with GPR41 in 2003. However, the studies about the role of GPR43 in disease are immensely larger. GPR43 is highly expressed on innate immune cells, specially neutrophils (17,80,114). Its activation appears to be regulated during inflammation by LPS or TNF- $\alpha$ , which have shown to raise GPR43 levels in human monocytes (5,141). Consistently, luciferase reporter assays have identified inflammation-associated NF $\kappa$ B transcription factor-binding sites within the GPR43 promoter (143).

SCFAs induce chemotaxis of neutrophils via GPR43 in p38, ERK and Akt-dependent manner (80,98,145,169) – see Figure 5. In fact, genetic and pharmacological inhibition of PI3K, p38 and ERK dramatically reduced the GPR43-dependent chemotaxis. In addition, this response is sensitive to pertussis toxin treatment, indicating the involvement of G $\alpha$ i in the process (169). However, recent studies have refuted this concept, reporting that GPR43 acts as an anti-inflammatory chemoattractant receptor in neutrophils in a model of IBD (98). Maslowski

et al. showed that mice lacking GPR43 presented exacerbated inflammation (more PMN recruitment and chemotaxis, higher ROS production, and increased expression levels of key inflammatory mediators such as CXCL1 or TNF- $\alpha$ ) in dextran sulphate sodium (DSS) - induced colitis, indicating that the GPR43-dependent effects of SCFAs are crucial for the normal resolution of intestinal inflammatory responses. Furthermore, greater intravascular neutrophil rolling and adhesion was observed in GPR43 knockout mice in response to LPS (61). Several contradictory results have been reported, about the role of GPR43 in IBD. Sina et al. reported that GPR43 deficient mice exhibited decreased inflammation in a DSS-induced colitis model, and in particular a reduced infiltration of neutrophils was observed (145). Thus, the question whether GPR43 exacerbates or reduces inflammation in the intestine remains unclear.

In the kidney, GPR43 activation by SCFAs or specific agonists has been shown to inhibit glomerular mesangial cell proliferation induced by high glucose and LPS. Receptor activation also reversed the production of ROS and malondialdehyde and increased expression levels of antioxidant enzymes. Likewise, GPR43 agonism also diminished the expression of adhesion molecules and pro-inflammatory cytokines such as MCP-1 and IL-1 $\beta$  from mesangial cells, suggesting a possible role of the receptor in diabetic nephropathy (53).

The action of SCFA/GPR43 signalling on inflammation and oxidative stress generation has been proposed to work through the Th17 response and NOX activation, respectively. Psoriatic mice treated with either acetate or a GPR43 agonist showed an increase in skin inflammation with upregulated Th17 immune responses and epidermal dual oxidase-2 signaling (105). But again, the effects of SCFA receptors on ROS production remain controversial. Some studies have shown a GPR43-mediated induction of ROS (98,110), whereas others have shown ROS inhibition (88,134,169).

GPR43 is expressed in intestine and adipose tissue, where it has been proposed to play a role in secretion of hormones and regulation of appetite (148). Interestingly, metabolic studies in GPR43 knockout mice appear to have consistent findings, consistent with GPR43 having a protective role against obesity and diabetes (73,100,159). GPR43 in enteroendocrine cells is involved in secretion of peptide YY (PYY), a hormone that decreases appetite (62,63). Additionally, it was demonstrated that GPR43 mediates SCFA-induced incretin hormone GLP-1 release in L enteroendocrine cells, thus indirectly participating in regulation of insulin



secretion and sensitivity (159). In adipose tissue, GPR43 regulates SCFA-mediated adipogenesis and adipocyte differentiation in mice (49).

Taken together, although there is much evidence showing that GPR43 and GPR41 are implicated in pathological metabolic and inflammatory processes, there are contradictory results about the beneficial or detrimental effects of activating these receptors in animal models. Furthermore, which direction of modulation (agonism or antagonism) would provide therapeutic effects is also under debate. The numerous discrepancies may be due to differences in the disease models used and/or non-specific gene knockout effects. Therefore, a further understanding and consensus will be necessary to launch GPR43 and GPR41 as pharmacologically valuable drug targets. Cell type specific knockout mice for GPR43 or GPR41 could be a useful approach to address this question as would specific small molecule modulators at these receptors (151).

#### **4. *Succinate receptor***

##### **4.1. *GPR91 (SUCNR1)***

In 2004 the biotechnology company Tularik published a landmark study that identified physiological ligands for not one, but two, orphan GPCR genes expressed at a high level in the kidney. Their experiments identified succinate as the major physiological agonist of GPR91 and  $\alpha$ -ketoglutarate as the agonist of GPR99. He et al. demonstrated that the citric acid cycle metabolite succinate is a full agonist at the human, mouse and rat GPR91 receptors expressed in transfected 293 cells and that signaling occurred partly via the pertussis toxin sensitive G $\alpha$ i pathway and via the pertussis toxin insensitive Gq pathway (47). Confirmation that succinate was physiologically active and that GPR91 was its unique receptor came from experiments performed using GPR91 knockout mice where administration of increasing doses of succinate raised mean arterial blood pressure via increased renin secretion in wild-type but not GPR91 knockout mice. Of note in this original description of GPR91 as a metabolite sensing GPCR was the high EC<sub>50</sub> of this receptor for succinate (low millimolar) compared to more traditional GPCRs previously shown to mediate hypertension and typical plasma levels of succinate (low micromolar). The authors of this original study suggested that pathophysiological relevance of succinate may lie in situations where blood supply to the kidney is restricted such as renal atherosclerosis or ischaemia. In 2014, Hamal et al.

demonstrated accumulation of succinate and functional activity of GPR91 in rodent models of cerebral post-hypoxia-ischemia revascularization (43). In the same year, Chouchani et al. identified a significant link between succinate and cardiac reperfusion injury using metabolomic approaches to demonstrate a link between succinate and mitochondrial ROS production but their work showed no role for extracellular succinate signaling via GPR91.

The ‘deorphanisation’ of GPR91 and the availability of GPR91 knockout mice led to multiple reports linking succinate and GPR91 with the dendritic cell biology (131), haematopoiesis (42), hepatic stellate cell activation in liver damage (23,84), lipolysis in adipose tissue (130) and retinal angiogenesis (135). The intriguing link between Krebs cycle intermediates and cell signaling that started with the pioneering work of He et al. has played an important role in the emerging concept of immunometabolism (47). O’Neill and co-workers have heralded succinate as a central player in the link between metabolism, ROS and innate immune cell activation in inflammation (102,115,157).

The causal links between GPR91 and hypertension, immunity and inflammation seen in the pre-clinical models outlined above lead to an obvious question – is the GPR91 succinate receptor a suitable drug target in human disease? Early work identified two orally bioavailable small molecule hGPR91 selective antagonists that were capable of blocking hypertensive responses to succinate infusion in rats (12). Taking as their starting point a report showing elevated levels of succinate in synovial fluid of rheumatoid arthritis patients (71), Littlewood-Evans et al. co-incubated activated human myeloid U937 cells with succinate or with supernatants of synovial fibroblasts from rheumatoid arthritis patients and showed that the GPR91 antagonist GPR91A1 reduced succinate induced macrophage IL-1 $\beta$  secretion in vitro (86). The authors then compared wild-type and Gpr91 knockout mice in an antigen induced arthritis model. Knee swelling was reduced 48% in Gpr91 deficient animals and this effect was shown to be conferred by bone marrow derived haematopoietic cells, most likely synovial monocyte-derived macrophages. This recent report suggests that GPR91 is a potential therapeutic target in rheumatoid arthritis due to the role of succinate in autocrine and paracrine amplification of macrophage inflammation – see Figure 6. Definitive evidence that GPR91 antagonists can act as novel anti-inflammatory drugs in vivo must await intervention studies in murine or rat inflammatory disease models and proof of concept studies in human volunteers.

## 5. *Niacin receptors*

Niacin also known as vitamin B3 and nicotinic acid has been used for over 50 years to treat dyslipidemia in patients at increased risk of atherosclerotic disease. Niacin in either an immediate release-or extended-release formulation has been shown to act on multiple cell types to reduce plasma LDL, decrease plasma triglyceride (TG), decrease plasma Lipoprotein (a) and elevate HDL – see Figure 7. These effects are rapid and significant, typically causing a 20-30% decrease in plasma lipids (19,20).

The GPR109A, GPR109B and GPR81 receptors are a genetically linked and highly related cluster of immunometabolic receptors. They are mainly expressed in adipocytes where they signal in response to metabolites including 3-hydroxybutyrate, lactate and 3-hydroxy-octanoate (1,120).

### 5.1. *GPR109A / HCA2 / HM74A/ PUMA-G*

In 2003 three groups independently demonstrated that GPR109A was a receptor for niacin and that GPR109A mediated niacin's anti-lipolytic effects in adipose tissue (149,162,173). Importantly, Gpr109a deficient mice showed no decrease in plasma FFA or TG following niacin treatment (162). An almost universal adverse effect of niacin is increased blood flow to the skin and associated skin inflammation and irritation – an effect known as 'flushing'. This significant adverse effect of niacin is mediated by GPR109A receptors expressed on keratinocytes and other non-haematopoietic cells in the skin. Prostaglandin synthesis in response to niacin causes Langerhans cell activation via prostaglandin D2 receptor 1 (DP1) (10,11,45) reviewed in (27). These observations prompted the development of a new formulation of niacin with a D1 receptor antagonist laropiprant to increase patient compliance.

In addition to its anti-atherogenic effects on plasma lipids, niacin has anti-inflammatory effects, which have been ascribed to GPR109A-expressing immune cells (118). Of note, monomethylfumarate (MMF), a metabolite of the anti-psoriasis and MS drug **Dimethyl fumarate**, was identified as a potent GPR109A agonist with an EC<sub>50</sub> of 9.4µM versus niacin 2.0µM in GPR109A transfected CHO cells (156). The tolerability of statins and their efficacy in lowering plasma LDL cholesterol and reducing the risk of cardiovascular events in multiple randomized clinical trials left clinicians with the question what to do with niacin. Early advice was to combine statins with niacin to effect even greater decreases in plasma

LDL. Fifty years after its first therapeutic use, niacin remains one of very few drugs that can increase anti-atherogenic HDL levels but whether this was of clinical value was unclear. For this reason, the results from two clinical trials AIM-HIGH and HPS2 THRIVE were eagerly awaited. The clear result from 25,673 vascular disease patients enrolled in the HPS study was that adding extended-release form of niacin and laropiprant to standard statin therapy did not significantly reduce cardiovascular disease events but did increase the risk of serious adverse drug effects (78). With the arrival of newer non statin LDL lowering agents such as ezetimibe and anti-proprotein convertase subtilisin/kexin type9 (PCSK9) drugs (15,33) there seems to be no use for niacin as a lipid lowering agent unless we can identify specific patient groups where there is a clear therapeutic benefit in activating the GPR109A receptor.

Consideration of the GPR109A literature, especially as it relates to pre-clinical models, suggests that the anti-inflammatory potential of GPR109A agonists remains under exploited. Early reports demonstrated niacin induction of the scavenger receptor CD36 and ATP-binding cassette transporter (ABCA1) expression in human myeloid cells likely via transcriptional activation of PPAR $\gamma$  and prostaglandin synthesis (74,132). More detailed consideration of disease mechanisms in Gpr109a $^{-/-}$  knockout mice in animal models of atherosclerosis have revealed interesting avenues for further study. Lukasova et al. reported that nicotinic acid (NA) was able to inhibit atherosclerosis in male Ldlr $^{-/-}$  mice but not Ldlr $^{-/-}$ , Gpr109a $^{-/-}$  double knockout mice. Importantly the anti-atherogenic effect of NA was not accompanied by any change in plasma cholesterol or HDL levels. The authors used bone marrow transplantation experiments to demonstrate that the anti-atherogenic effect of NA was associated with radiosensitive GPR109A expressing haematopoietic cells. Intriguingly the authors demonstrated that NA treatment inhibited macrophage recruitment to the peritoneum following MCP-1 injection into wild-type but not Gpr109A $^{-/-}$  mice. This finding was corroborated by following the recruitment of fluorescently labeled macrophages into Ldlr $^{-/-}$  mouse atherosclerotic lesions of NA treated mice (92).

It is not immediately obvious how GPR109A signalling interferes with chemokine recruitment of monocytes and macrophages. Understanding exactly how GPR109A agonists interfere with monocyte chemoattraction in vivo and in vitro might lead to new anti-inflammatory strategies (183). In a subsequent paper Lauring et al. studied the effect of niacin on atherosclerosis and lipid lowering in Ldlr $^{-/-}$  mice expressing the human cholesterol ester transfer protein (CETP). Lauring et al concluded that in their animal model of atherosclerosis the GPR109A receptor is not responsible for the beneficial lipid effects of niacin (79).

A subsequent study by Singh et al. revealed a subtle cellular phenotype in Gpr109A<sup>-/-</sup> mice, with 40% fewer IL-10 producing CD4<sup>+</sup> T cells and more IL-17A producing CD4<sup>+</sup> T cells present in the colon (146). Interestingly Gpr109A<sup>-/-</sup> colonic epithelium showed a defect in expression of the cytokine IL-18 providing evidence of a potential link between the niacin receptor and inflammasome activation. The importance of these cellular changes was demonstrated by increased colonic inflammation when Gpr109A<sup>-/-</sup> mice were used in the DSS colitis model and increased polyp formation when mice were dosed with azoxymetane (AOX) and DSS.

For a drug used for over 50 years in the treatment of human cardiovascular disease, niacin and its G protein coupled receptor GPR109A remain enigmatic when it comes to mechanism, not least the mechanistic basis of the anti-atherogenic and anti-inflammatory effects. Nevertheless, the HPS THRIVE randomized clinical trial showed that no additional therapeutic benefit was derived from the addition of niacin to statins in humans and there is little current enthusiasm to identify novel GPR109A agonists as a new class of anti-inflammatory drugs.

## **5.2. *GPR109B / HCA3***

In vitro experiments have shown that GPR109A and GPR109B heterodimerise in transfected HEK cells but so far there is no evidence for altered signaling by heterodimers or signal induced heterodimerisation (95). Irukayama-Tomode et al. reported GPR109B (HM74) activation by aromatic D-amino acids but not niacin, and expression by of GPR109B mRNA by human neutrophils, but they did not directly demonstrate GPR109B-mediated leukocyte chemotaxis of primary leukocytes (56). Liu et al. demonstrated differential G protein coupling of the C-terminus tails of the HCA2 and HCA3 in yeast (89) but a clearly differentiated physiological role for the GPR109B receptor over its GPR109A neighbour has yet to emerge (119).

## **5.3. *GPR81 / HCA1 / HCARI***

The GPR81 lactate receptor is closely related in amino acid sequence to the GPR109A receptor and is expressed predominantly in adipocytes (18,38). Lactate signaling in WAT via the GPR81 receptor decreases lipolysis. A recent study by Sakurai et al identified a series of novel GPR81 agonists that can suppress lipolysis in differentiated 3T3-L1 adipocytes and explanted WAT. These GPR81 agonists can reduce lipolysis in vivo without flushing when

administered intraperitoneally in mice (162). Lactate is known to exert anti-inflammatory effects on murine macrophages in vitro but these effects were shown to be independent of the murine GPR81 receptor by using bone marrow derived macrophages from wild-type and Gpr81<sup>-/-</sup> mice (31). At first sight this study seems somewhat at odds with a previous report showing that intraperitoneal delivery of both anti GPR81 siRNA and lactate reduced LPS induced hepatitis and pancreatitis (50,83). One explanation for this discrepancy may lie in the use of siRNA knockdown technology rather than genetic knockout of the Gpr81 gene. It is important to note that not all the physiological effects of lactate are mediated by GPR81 so it will be important to use cell type specific GPR81 knockout animals and GPR81 selective agonists and antagonists in pre-clinical models of inflammation before extending studies into human (patho) physiology.

### **Important questions and future challenges**

GPCRs were well known to have important roles in endocrine and metabolic functions but recent research has highlighted their importance in inducing or balancing inflammatory and immune responses. Thus select GPCRs are emerging as important players in the new field of immunometabolism. In this section, we present a list of questions to be addressed by the immunometabolism research community over the next 5 to 10 years and we identify current technical hurdles that limit our understanding of immunometabolic GPCR signalling in inflammation and oxidative stress. We need a better understanding of the basic science of this class of signalling receptors to enable access to a wider range of highly selective bioavailable small molecule tool compounds, to improve drug development and inform clinical trial design.

#### ***1. DRG TO COMPLETE***

***2. Are all cells of the innate and adaptive immune response subject to immunometabolic control?*** RNA expression data for the GPCRs reviewed here suggests higher mRNA expression in innate immune cells compared to adaptive immune cells. Furthermore, innate immune cell gene expression can be further enhanced by inflammatory cytokines and TLR ligands (ImmGen Consortium and Carlota Recio et al., unpublished data). However, it is not enough to measure relative mRNA abundance. There is a pressing need to develop specific antibody reagents to unambiguously detect cell surface expression of all the

GPCRs we have reviewed here. Just looking at mRNA expression levels or detecting fluorescent transgene expression cannot be relied upon to identify cells that are capable of responding to specific metabolites via GPCRs.

**3. *Can metabolites working through the GPCRs reviewed here significantly affect macrophage differentiation and macrophage function in vivo?*** It will be important to critically assess macrophage biology in a range of different tissues, including adipose tissues and atherosclerotic lesions. An area that remains almost completely unexplored remains the effect of metabolites on microglial cell biology. A recent publication reported effects of microbiota and plasma acetate levels on murine microglia morphology but important functional consequences of SCFA receptor activation in the central nervous system remain to be demonstrated and could be very important in developmental (e.g autism) and degenerative (e.g. **Alzheimer's**) neurological disease.

**4.** Immunometabolism is clearly linked to changes in diet and microbiota composition and this raises the important question - ***Can dietary manipulation in humans drive useful changes in innate and adaptive immune responses?*** Current advice to switch to a diet containing more fibre is unlikely to cause harm but exactly how does the combination of a diet rich in fibre and a 'healthy' microbiota result in less 'low grade' inflammation and a 'healthier' immune response. It is possible that we can accelerate or stabilise these changes using allosteric agonists active at immunometabolic receptors. It is interesting to note that many of the compounds active at the FFARs are ago-allosteric modulators (101).

**5. *Can we develop a wider range of metabolic sensing GPCR tool compounds with drug-like properties?*** New tool compounds could be used to critically assess the role of candidate GPCRs in metabolic disease and chronic inflammation processes in vitro and in vivo. Some of the GPCRs we have discussed here (e.g. GPR120) have been the subject of multiple commercial drug development programmes but details of the studies performed are largely unpublished and access to useful tool compounds is limited. For other GPCRs (e.g. GPR84) there are only a very limited number of structurally distinct tool compounds known (see Figure 4) and few if any antagonists or partial agonists are available in the public domain. A range of tool compounds that display biased agonism would allow us to test the full range of specific GPCR signalling modalities. GPR84 also illustrates a potentially common theme for all the GPCRs discussed in this review, do we really know what the physiologically relevant agonists are at these immunometabolic receptors?

## **6. *Do metabolite sensing GPCRs mediate leukocyte homing and chemotaxis in vivo?***

Of note, most of the immunometabolic GPCRs we have discussed are G $\alpha$ i coupled receptors and this is suggestive of a role in leukocyte migration. There are hints in the published literature that metabolites or immunometabolic receptors such as GPR109A can modulate monocyte / macrophage recruitment in vivo. The development of cell type specific GPCR knockout animals will be very useful to test the hypothesis that metabolites, signalling via specific GPCRs, can act as important leukocyte chemoattractants in vivo.

In summary the family of GPCRs that signal in response to endogenous metabolites and/or microbial fermentation products represent a fascinating entry point into the world of immunometabolism. Over the next 5 years the development of new transgenic mice, receptor-specific monoclonal antibodies and a comprehensive panel of GPCR tool compounds will allow us to identify which of these receptors play a non-redundant role in regulating host immunity and host metabolism in health and disease.

### ***DRG TO COMPLETE***

#### **Abbreviations used**

6-OAU6-n-octylaminouracil

ABCA1      ATP-binding cassette transporter A1

ALA       $\alpha$ -linolenic acid

AOX      azoxymetane

cAMP      cyclic AMP

CETP      cholesterol ester transfer protein

CLA      conjugated linoleic acid

DAG      diacylglycerol

DBA      Dilute Brown Non-Agouti



DHA	docosahexaenoic acid
DIM	diindolylmethane
DP1	prostaglandin D2 receptor 1
DSS	dextran sulphate sodium
Duox	dual oxidases
EAE	experimental autoimmune encephalomyelitis
EPA	eicosapentaenoic acid
ER	endoplasmic reticulum
FFA	free fatty acid
GIP	gastric inhibitory polypeptide
GLP-1	glucagon-like peptide-1
GPCR	G protein-coupled receptor
GSHPx	glutathione peroxidase
HFD	high fat diet
IBD	inflammatory bowel disease
IL	interleukin
IP3	inositol triphosphate
IR	insulin receptor
LCFA	long chain fatty acid
LDL	low-density lipoprotein
LPS	lipopolysaccharide
MCFA	medium chain fatty acid
MMF	monomethylfumerate

MMP-9	metalloproteinase-9
mRNA	messenger RNA
NA	nicotinic acid
NAD(P)H	nicotinamide adenine dinucleotide phosphate
NFκB	nuclear factor kappa-light-chain-enhancer of activated B cells
NOX	NAD(P)H oxidase
PIP2	phosphatidylinositol 4,5-bisphosphate
PKC	protein kinase C
PMN	polymorphonuclear
PPARγ	peroxisome proliferator activated receptor gamma
PSCK9	proprotein convertase subtilisin/kexin type9
PYY	peptide YY
ROS	reactive oxygen species
SCFA	short chain fatty acid
SOD	superoxide dismutase
T2D	type 2 diabetes
TG	triglyceride
TLR4	toll like receptor-4
TNF-α	tumour necrosis factor-α
WAT	white adipose tissue
ω3-PUFA	omega-3 fatty acid

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## Figure and Table Legends

**Table 1.** Metabolite-sensing GPCRs involved in immunometabolic signalling

**Figure 1.** Illustrative flowchart that represents the role that inflammation and oxidative stress play in metabolic disease.

Activation of intracellular signalling pathways aggravates the inflammatory process leading to severe consequences such as insulin resistance (T2D) and increased risk of CVD.

**Figure 2.** General structure of GPCRs. *Top left:* the snake diagram shows the seven transmembrane domains with the extracellular N-terminus and the C-terminus located inside the cell. *Top right:* proposed molecular structure of a GPCR in complex with  $G\alpha\beta\gamma$  (taken from the studies of Brian Kobilka and Robert Lefkowitz in GPCRs that gave them the Nobel Prize in Chemistry in 2012 (9)). The representation shows the seven transmembrane helices which are arranged in an approximately circular fashion within the membrane plane. The yellow structure represents the GPCR ligand within the binding site which is in the middle of the helices, being accessible from the extracellular side. *Bottom panel:* different G protein coupling of the metabolite sensing GPCRs included in this review.

**Figure 3.** Proposed mechanism of action of long-chain fatty acid receptors (GPR120 and GPR40) in different cell types.

**Top left panel:** Both GPR40 and GPR120 are expressed in immune cells and signal via different intracellular cascades including TLR4, TNF $\alpha$ , JNK and IKK involved in inflammation and migration. **Top middle panel:** LCFA-induced GPR120 signaling elicits adipogenesis and increased glucose uptake in adipose tissue. **Top right panel:** GPR40 and GPR120 regulate the secretion of GLP-1 in enteroendocrine cells. **Bottom left panel:** In pancreatic cells, both GPR40 and GPR120 promote LCFA-mediated insulin secretion. **Bottom right panel:** GPR40 is expressed in neurons exerting a neuroprotective role and decreasing nociception.

**Figure 4.** Chemical structures of reported GPR84 physiological agonists and GPR84 tool compounds.

**Figure 5.** SCFA receptor GPR43 proposed mechanism of action in neutrophils.

SCFA-induced GPR43 signaling elicits chemotaxis and increased inflammatory profile in neutrophils by activating different intracellular signalling pathways including MAPK kinases, ERK and p38.

**Figure 6.** Succinate receptor proposed mechanism of action.

Succinate-mediated GPR91 signalling (red arrows) activates inflammatory cascades in macrophages that lead to tissue inflammation. GPR91-driven increased glycolysis (blue arrows) promotes the synthesis of succinate that exerts both autocrine effects in macrophages and paracrine effects on neighbouring cells. Modified from Littlewood-Evans et al. (86).

**Figure 7.** Niacin receptors mechanism of action.

**Left part:** In adipocytes, nicotinic acid binds niacin receptors leading to a decrease in free fatty acids mobilization from adipose tissue to the liver resulting in a reduction of TG and thus LDL and VLDL synthesis. **Right part:** In macrophages and microglia, niacin receptor-

mediated signalling induces antioxidant and neuroprotective effects and reduces inflammation.

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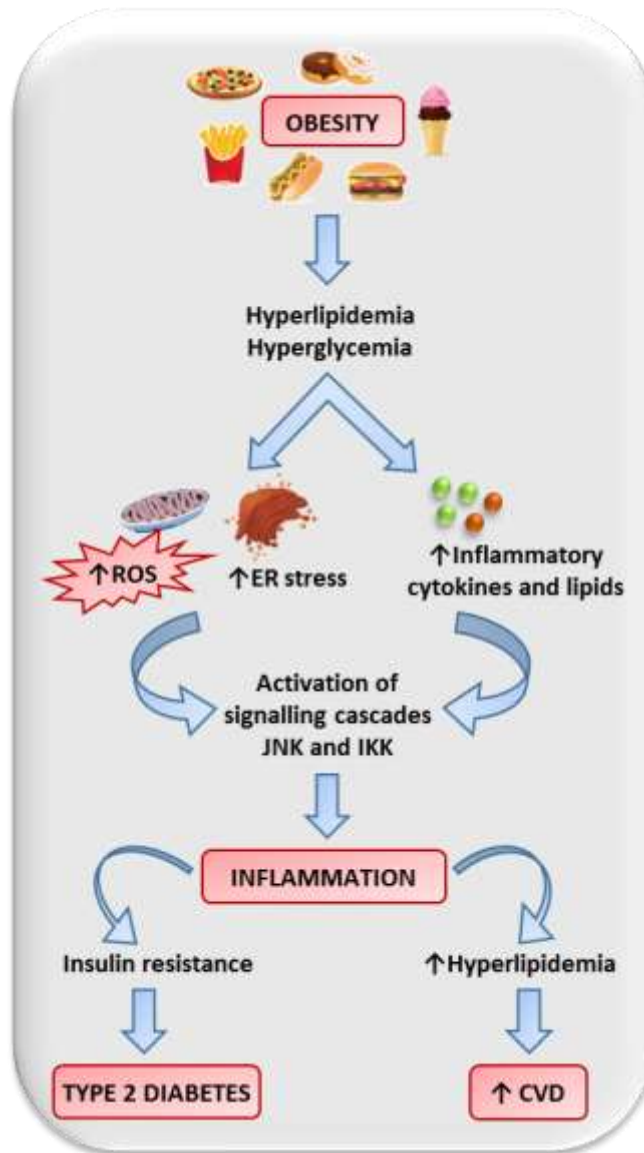
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**Table 1.** Metabolite-sensing GPCRs involved in immunometabolic signalling

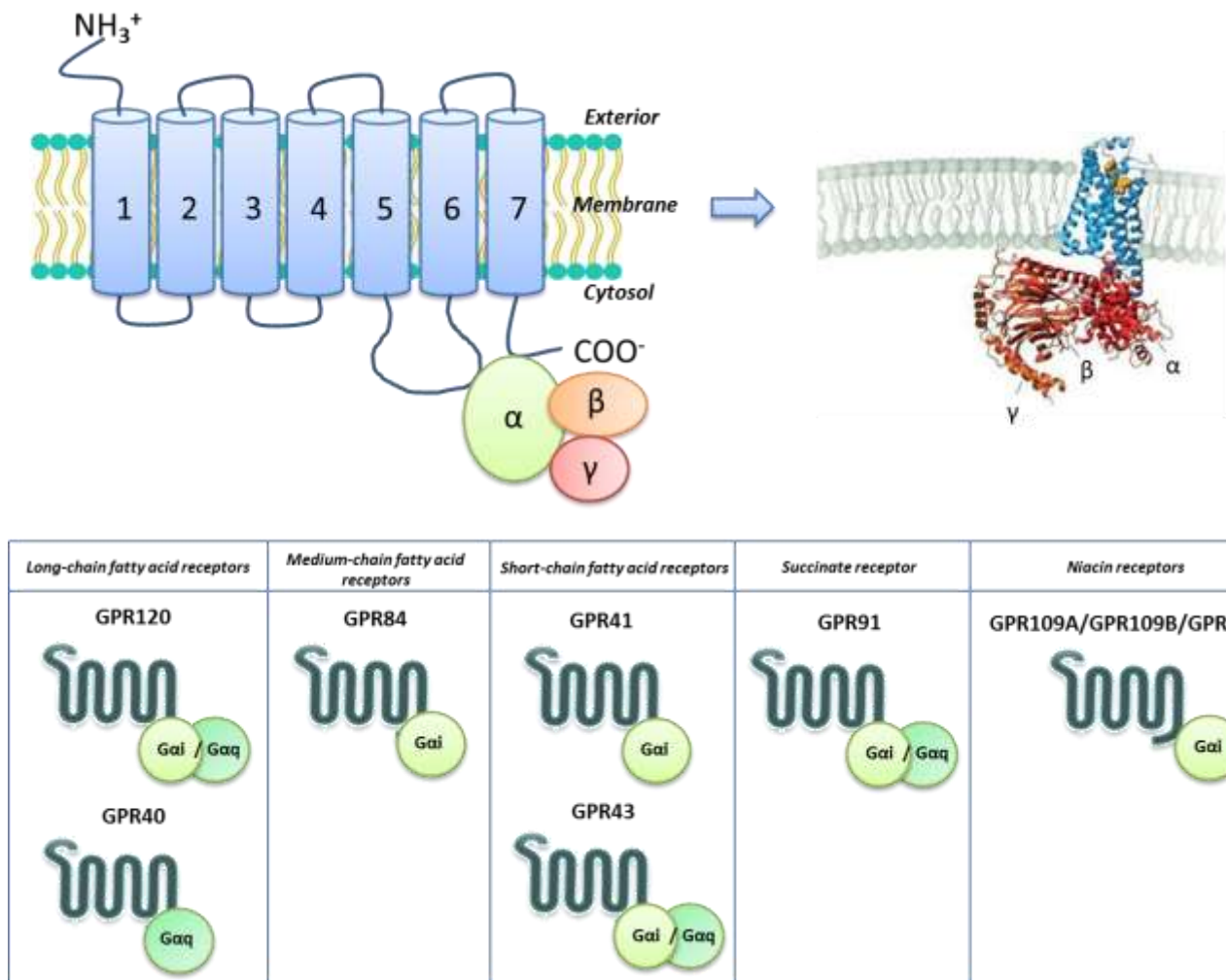
<i>Receptor</i>	<i>Metabolite Ligands</i>	<i>G protein coupling</i>	<i>Expression</i>
<i>Long-chain fatty acid receptors</i>			
<b>GPR120 (FFAR4)</b>	LCFAs (C12-C22): unsaturated, $\omega$ -3 and $\omega$ -6 fatty acids	$G_{\alpha i}/G_{\alpha q}$	Immune cells: macrophages Intestine enteroendocrine cells Pancreatic cells Adipocytes
<b>GPR40 (FFAR1)</b>	LCFAs (C12-C22), MCFAs (C7-C12), and CLAs.	$G_{\alpha q}$	Pancreatic $\beta$ cells Enteroendocrine cells Immune cells: neutrophils Neurons Taste buds cells
<i>Medium-chain fatty acid receptors</i>			
<b>GPR84 (EX33)</b>	MCFAs (C2-C6): Capric acid, decanoic acid and undecanoic acid.	$G_{\alpha i}$	Immune cells: leukocytes, mainly macrophages and microglia cells
<i>Short-chain fatty acid receptors</i>			
<b>GPR43 (FFAR2)</b>	SCFAs (C2-C6): Acetate, Propionate, Butyrate	$G_{\alpha i}/G_{\alpha q}$	Immune cells: neutrophils and eosinophils Enteroendocrine cells Adipocytes Pancreatic islet cells
<b>GPR41 (FFAR3)</b>	SCFAs (C2-C6): Acetate, Propionate, Butyrate	$G_{\alpha i}$	Immune cells: peripheral blood mononuclear cells and macrophages Enteroendocrine cells Pancreatic islet cells Neurons
<i>Succinate receptors</i>			
<b>GPR91 (SUCNR1)</b>	Succinate	$G_{\alpha i}/G_{\alpha q}$	Immune cells: dendritic cells, macrophages and platelets Kidney: macula densa and endothelial cells Adipocytes Retinal neurons Other tissues: liver, heart, intestine and spleen
<i>Niacin receptors</i>			
<b>GPR109A (HCA2/NIACR1)</b>	3-hydroxybutyrate and SCFAs: butyrate	$G_{\alpha i}$	Immune cells: macrophages, dendritic cells Adipocytes Langerhans cells Intestinal epithelial cells Keratinocytes
<b>GPR109B (HCA3/NIACR2)</b>	3- hydroxyoctanoate	$G_{\alpha i}$	Immune cells: macrophages, neutrophils Adipocytes Colon epithelial cells
<b>GPR81 (HCA1)</b>	Lactate	$G_{\alpha i}$	Adipocytes



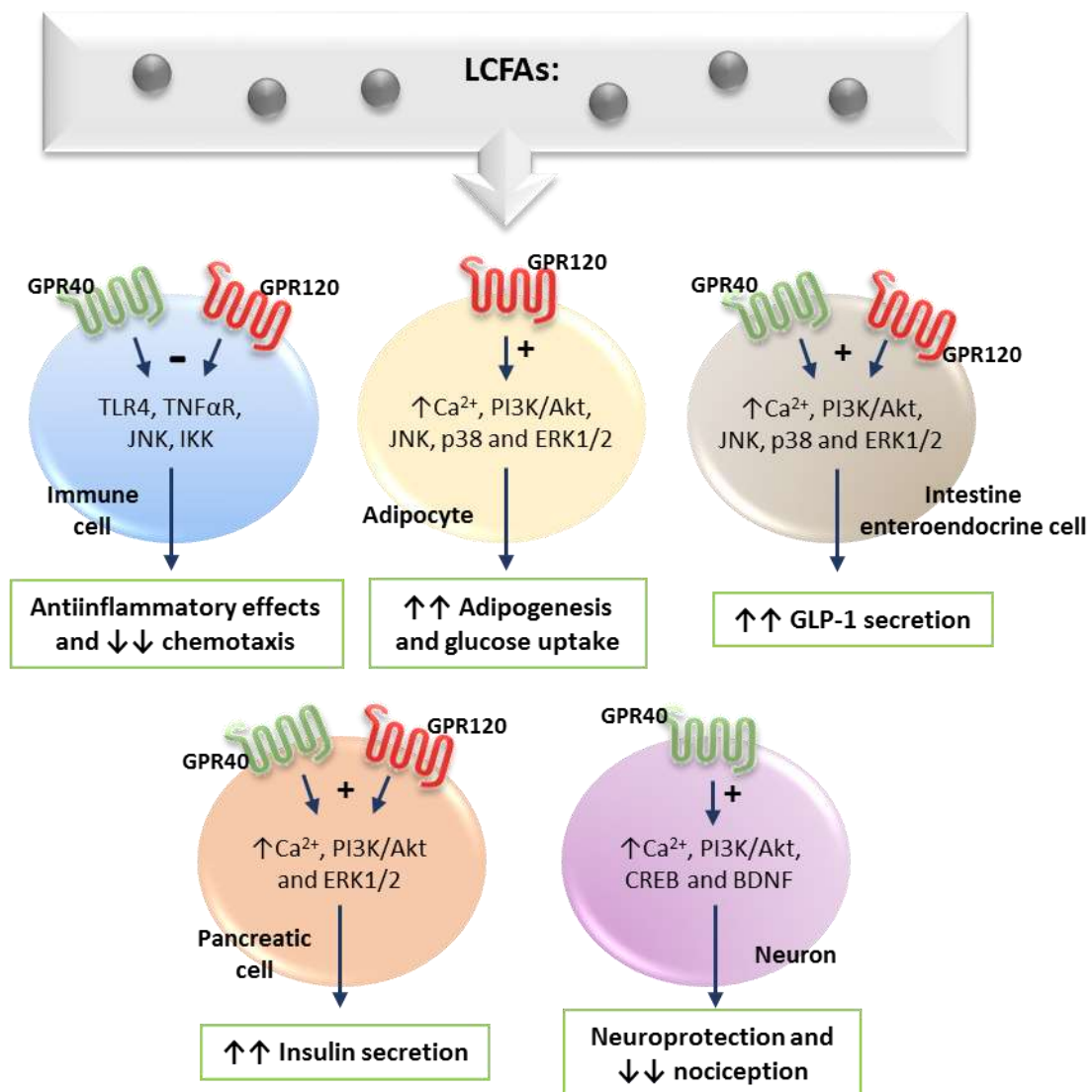
**Figure 1.** Illustrative flowchart that represents the role that inflammation and oxidative stress play in metabolic disease.

Activation of intracellular signalling pathways aggravates the inflammatory process leading to severe consequences such as insulin resistance (T2D) and increased risk of CVD.



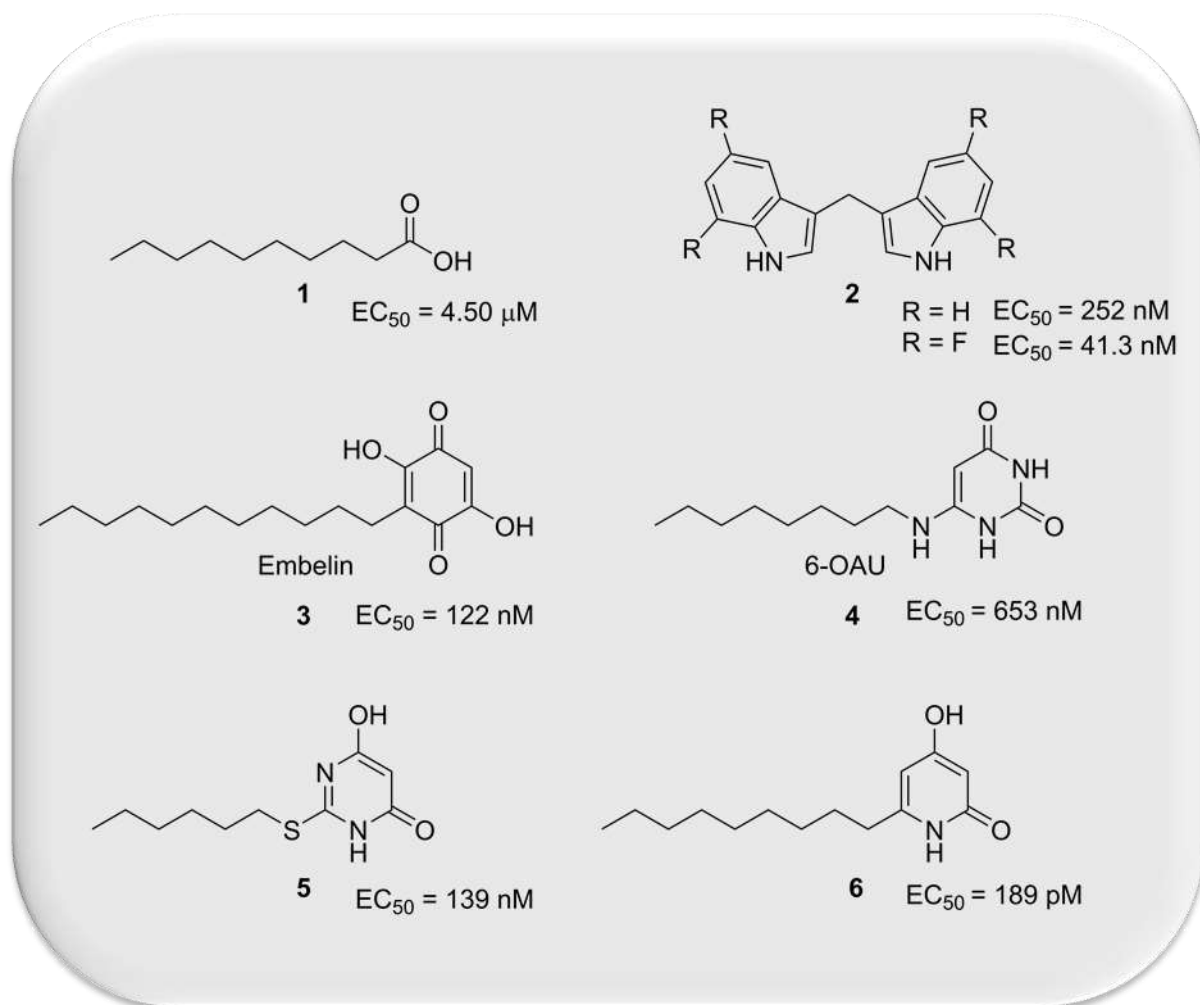


**Figure 2.** General structure of GPCRs. *Top left:* the snake diagram shows the seven transmembrane domains with the extracellular N-terminus and the C-terminus located inside the cell. *Top right:* proposed molecular structure of a GPCR in complex with  $G\alpha\beta\gamma$  (taken from the studies of Brian Kobilka and Robert Lefkowitz in GPCRs that gave them the Nobel Prize in Chemistry in 2012 (9)). The representation shows the seven transmembrane helices which are arranged in an approximately circular fashion within the membrane plane. The yellow structure represents the GPCR ligand within the binding site which is in the middle of the helices, being accessible from the extracellular side. *Bottom panel:* different G protein coupling of the metabolite sensing GPCRs included in this review.

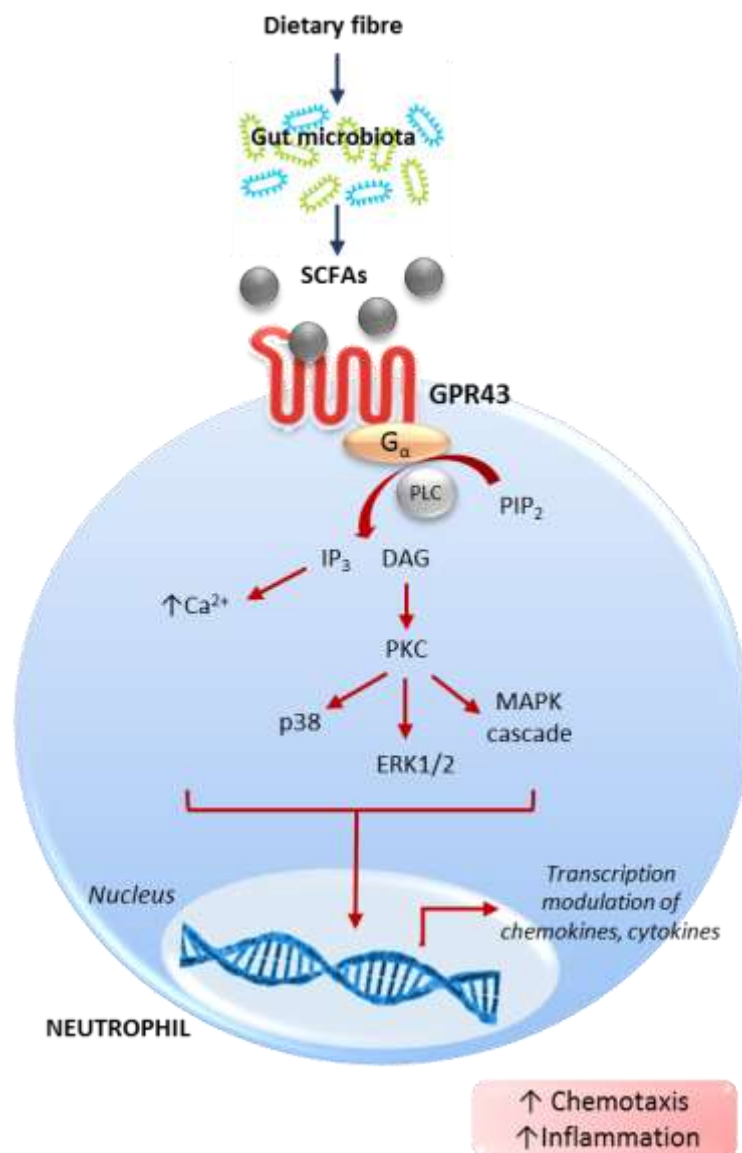


**Figure 3.** Proposed mechanism of action of long-chain fatty acid receptors (GPR120 and GPR40) in different cell types.

**Top left panel:** Both GPR40 and GPR120 are expressed in immune cells and signal via different intracellular cascades including TLR4, TNF $\alpha$ , JNK and IKK involved in inflammation and migration. **Top middle panel:** LCFA-induced GPR120 signaling elicits adipogenesis and increased glucose uptake in adipose tissue. **Top right panel:** GPR40 and GPR120 regulate the secretion of GLP-1 in enteroendocrine cells. **Bottom left panel:** In pancreatic cells, both GPR40 and GPR120 promote LCFA-mediated insulin secretion. **Bottom right panel:** GPR40 is expressed in neurons exerting a neuroprotective role and decreasing nociception.

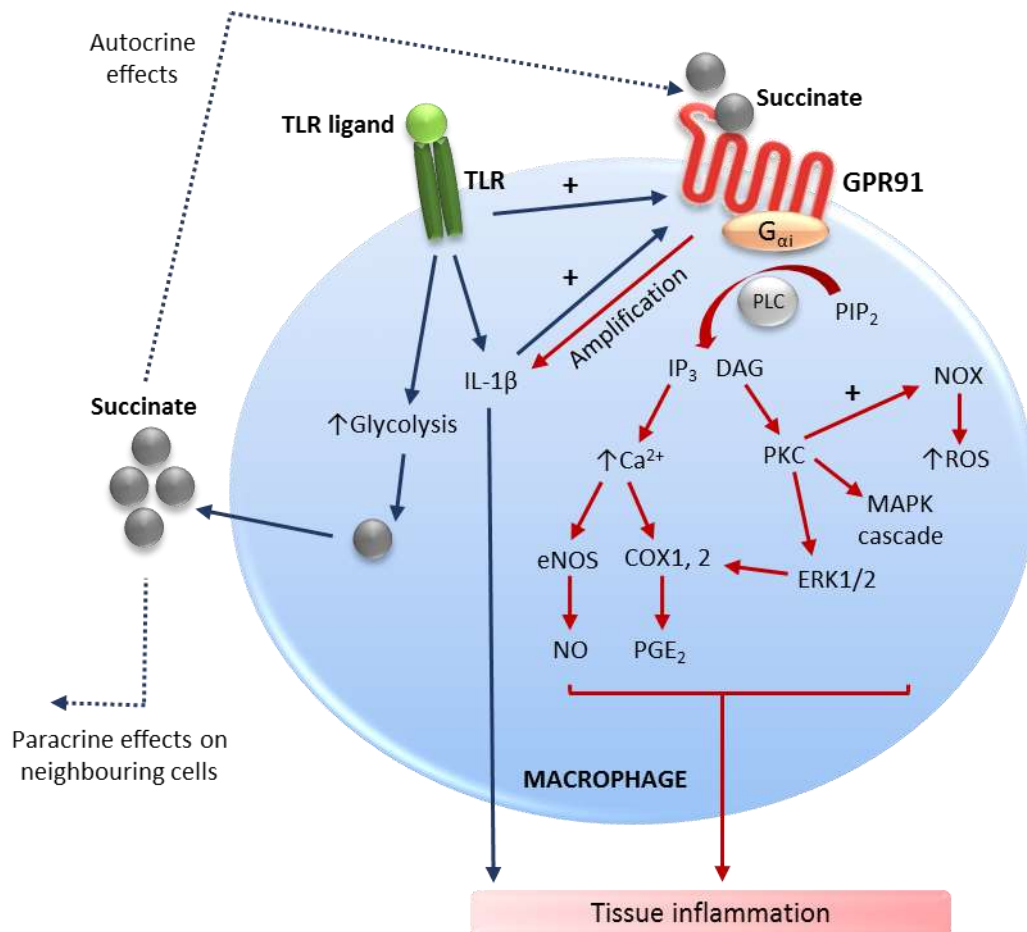


**Figure 4.** Chemical structures of reported GPR84 physiological agonists and GPR84 tool compounds.



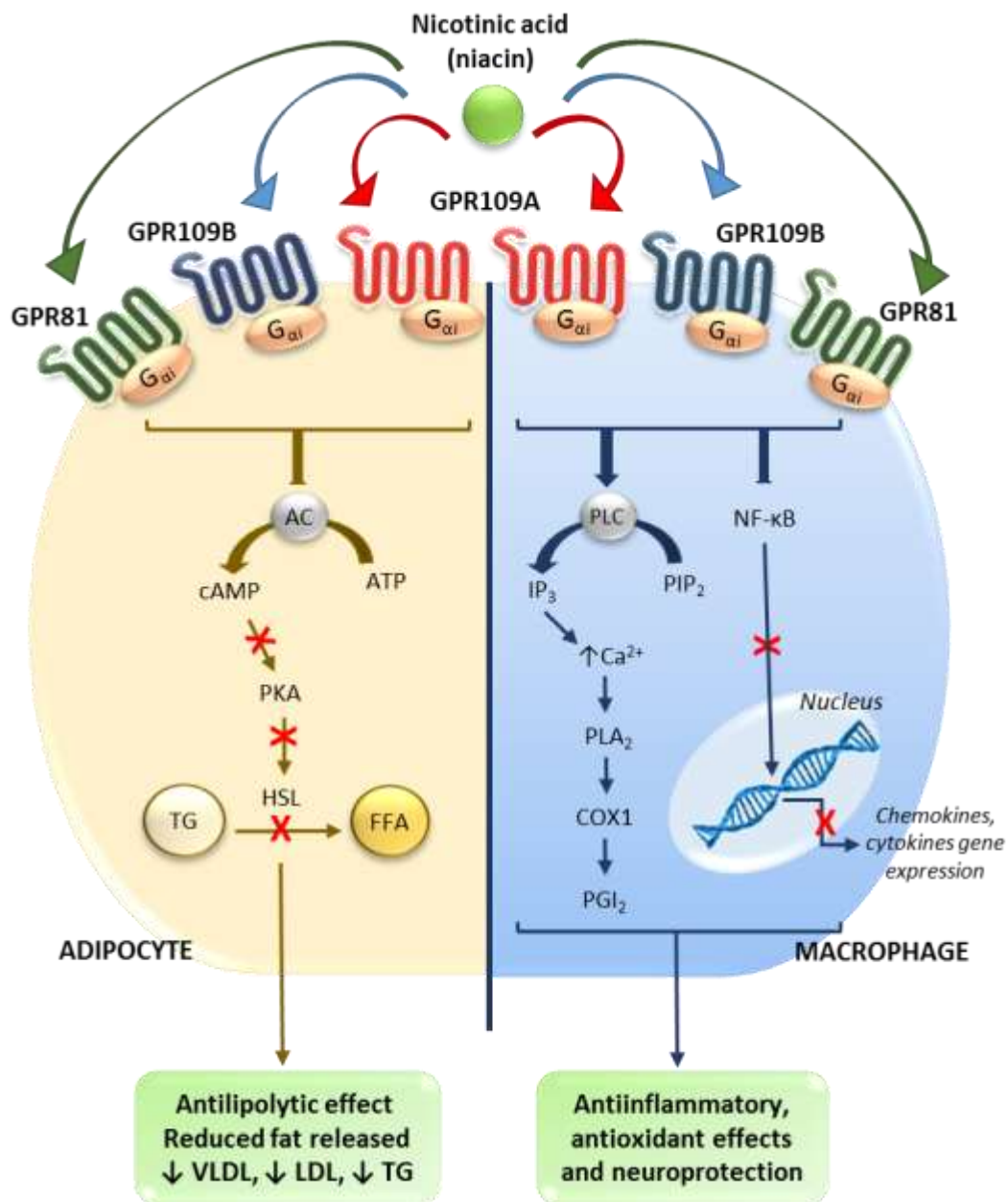
**Figure 5.** SCFA receptor GPR43 proposed mechanism of action in neutrophils.

SCFA-induced GPR43 signaling elicits chemotaxis and increased inflammatory profile in neutrophils by activating different intracellular signalling pathways including MAPK kinases, ERK and p38.



**Figure 6.** Succinate receptor proposed mechanism of action.

Succinate-mediated GPR91 signalling (red arrows) activates inflammatory cascades in macrophages that lead to tissue inflammation. GPR91-driven increased glycolysis (blue arrows) promotes the synthesis of succinate that exerts both autocrine effects in macrophages and paracrine effects on neighbouring cells. Modified from Littlewood-Evans et al. (86).



**Figure 7.** Niacin receptors mechanism of action.

**Left part:** In adipocytes, nicotinic acid binds niacin receptors leading to a decrease in free fatty acids mobilization from adipose tissue to the liver resulting in a reduction of TG and thus LDL and VLDL synthesis. **Right part:** In macrophages and microglia, niacin receptor-mediated signalling induces antioxidant and neuroprotective effects and reduces inflammation.