Review

How Arrestins and GRKs Regulate the Function of Long Chain Fatty Acid Receptors

Abdulrahman G. Alharbi¹,², Andrew B. Tobin¹ and Graeme Milligan¹,∗

¹ Centre for Translational Pharmacology, School of Biomolecular Sciences, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK
² Department of Pharmacology and Toxicology, College of Pharmacy, Taibah University, Madinah 42353, Saudi Arabia
∗ Correspondence: graeme.milligan@glasgow.ac.uk

Abstract: FFA1 and FFA4, two G protein-coupled receptors that are activated by long chain fatty acids, play crucial roles in mediating many biological functions in the body. As a result, these fatty acid receptors have gained considerable attention due to their potential to be targeted for the treatment of type-2 diabetes. However, the relative contribution of canonical G protein-mediated signalling versus the effects of agonist-induced phosphorylation and interactions with β-arrestins have yet to be fully defined. Recently, several reports have highlighted the ability of β-arrestins and GRKs to interact with and modulate different functions of both FFA1 and FFA4, suggesting that it is indeed important to consider these interactions when studying the roles of FFA1 and FFA4 in both normal physiology and in different disease settings. Here, we discuss what is currently known and show the importance of understanding fully how β-arrestins and GRKs regulate the function of long chain fatty acid receptors.

Keywords: arrestins; GRKs; FFA1; FFA4

1. Introduction: Free Fatty Acids (FFAs)

There has recently been a substantial expansion of knowledge concerning the ability of chemicals found in, or generated from, dietary sources to manage and govern cellular activity and maintain homeostasis [1–3]. Amongst such molecules non-esterified or “free” fatty acids (FFAs) have long been recognised as having various impacts on numerous biological systems, including cardiovascular health, metabolism, and inflammation [4]. Although it was previously thought that fatty acids exerted such actions exclusively via intracellular targets, it has subsequently been shown that they also stimulate a number of cell surface G protein-coupled receptors (GPCRs). It has long been a staple of drug development efforts to target GPCRs [5] and therefore GPCRs activated by FFAs have become of major interest as potential novel therapeutic targets [6–8]. Indeed, programmes targeting the receptors for FFAs have investigated the actions of each of orthosteric and allosteric modulators to try to optimise therapeutic activity [9,10]. Major efforts have examined these receptors in relation to metabolism and inflammation, in particular the role of FFA1 and FFA4 in the management of type-2 diabetes [7,8,11,12]. Other studies have also indicated that they play roles in areas ranging from lung function to the central nervous system [13–15]. Currently four GPCRs, FFA1–FFA4, are considered as authentic free fatty acid receptors, and whilst GPR84 remains officially an orphan receptor [16], it is nevertheless widely known that medium chain length (C9–C12) fatty acids are able to activate this receptor, indicating it to be a further FFA responsive GPCR. Short chain fatty acids (C2–C6) are selective activators of FFA2 and FFA3 whilst longer chain fatty acids are effective agonists for FFA1 and FFA4 [4,17,18]. Both FFA1 and FFA4 are extensively expressed in a variety of tissues and regions of the body and participate in vital physiological...
functions that are closely connected to metabolism and immunity [12] (Figure 1). Many pharmaceutical companies have initiated studies to design and test ligands that stimulate FFA1 and FFA4 due to the role of these receptors in the control of blood glucose with the ambition to treat the increasing number of T2D patients. The focus of this review will hence be on FFA1 and FFA4, the receptors for longer chain fatty acids, and on how their function is regulated by GPCR kinases (GRKs) and by arrestins.

![Figure 1. Activities that may be regulated by FFA1 and FFA4 receptor ligands.](image)

Free Fatty Acid Receptor 1

Still frequently referred to as GPR40, the Free Fatty Acid Receptor 1 (FFA1), which was first described merely as an uncharacterised GPCR sequence found in humans at chromosome location 19q13.1, is expressed in high levels by pancreatic β-cells. Three distinct investigations reported in 2003 showed this receptor could be stimulated by medium and long chain fatty acids [19–21]. A variety of saturated and unsaturated fatty acids activate the receptor and results in an increase in intracellular Ca^{2+} levels. Several studies employed the selective G_q/11 inhibitor YM-254890 [22] to establish that such signals from FFA1 stimulation are transmitted mostly through G_q/11-family G proteins [23]. Recent years have also seen the use of FR900359 [24], a second, related depsipeptide G_q/11 inhibitor, to corroborate these findings. Many FFA1-active ligands have been developed and reported (Table 1), and a range of studies have been conducted in relation to the potential treatment of many physiological and metabolic illnesses, including cardiovascular diseases, type-2 diabetes, obesity, atherosclerosis, ulcerative colitis, Crohn's disease, and irritable bowel syndrome [25,26]. Among these disorders, the activity of FFA1 in promoting the release of insulin from pancreatic β-cells in a glucose concentration-dependent manner has attracted the greatest attention [27,28]. At least in part this effect on insulin release reflects receptor-induced enhancement of intracellular calcium signalling [29–31] whereby
intracellular Ca\(^{2+}\) levels rise as a result of G\(_q/G_{11}\)-mediated production of the secondary messenger inositol 1,4,5 trisphosphate (IP3). However, glucose-stimulated insulin secretion (GSIS) from pancreatic β-cells not only requires the presence of glucose but also requires other signalling stimuli that are activated by external calcium [20,32,33]. Examples of these stimuli include peptide hormones, neurotransmitters, and other compounds [8]. Although G protein-dependent pathways are recognised to be the primary mechanism by which FFA1 mediates signal transduction [28], there is nevertheless another signalling route that FFA1 may activate (Figure 2), where function is reliant on arrestin adapter proteins [34]. Indeed insulin secretion is connected to an FFA1-arrestin signalling axis [35].

![Figure 2. Major FFA1 signalling pathways](image)

Both orthosteric and allosteric FFA1 agonists have been identified and developed and these may differentially regulate FFA1 activity, resulting in a signalling bias [35]. Fatty acids, such as palmitic acid (C16:0) and oleic acid (C18:1), generate G\(_q/G_{11}\)-mediated signalling via FFA1; however, they also recruit β-arrestin 2 and promote internalisation of FFA1 from the cell surface [34]. β-Arrestin recruitment to FFA1 originates via both G\(_q/G_{11}\)-dependent and -independent processes with the balance between these functions induced by distinct activators that may define their effectiveness [34]. For example, both palmitic acid and oleic acid have insulinotropic effects, mostly due to G\(_q/G_{11}\) mediated pathways. However, the clinically trialled synthetic allosteric agonist, TAK-875 demonstrated a distinct relative efficacy for activating the G\(_q/G_{11}\) and β-arrestin routes. Here it is only a partial agonist for G\(_q/G_{11}\) activation whilst the fatty acids palmitate and oleate may be less efficient than
TAK-875 for the recruitment of β-arrestins [34]. To improve insulin production in people with type-2 diabetes, targeting the biased stimulation of FFA1 has shown promising results [15]. However phase III clinical trials with TAK-875 were terminated because of indications of liver toxicity in patients [36]. As a result, although treatments based on FFA1 agonism may still provide a promising option in the search for new anti-diabetic medications, and a number of additional ligands have already been assessed in rodent models, there has nevertheless been limited progression towards human studies beyond initial phase I safety studies (e.g., [37]). Importantly, recent research has revealed the remarkable complexity of FFA1 ligand actions providing optimism that with a better knowledge of the underlying processes the next phase will be more effective [29,38]. Additional studies include phase I data on the oral FFA1 inhibitor P11187 [12] but, whilst completing phase II clinical trials, the development of JTT-851, a further orally active FFA1 agonist, was also terminated [39]. Further molecules that have shown interesting characteristics in rodent models include the high potency FFA1 agonist TUG-770, which was able to increase glucose tolerance in high fat diet fed mice [40] and DS-1558 which improved insulin production and glucose homeostasis in ‘Zucker’ diabetic fatty rats [40]. Other compounds such as AM-1638 and AM-5262 can additionally engage further signalling cascades including the Gαi G protein [41–43]. These compounds enhance incretin production from enteroendocrine cells and directly stimulate insulin production whilst AP1 and AP3, both of which are reported as full agonists of FFA1, promote insulin and incretin production in diabetic ‘Goto-Kakizaki’ rats, resulting in a reduction in both body mass and blood sugar levels [43,44]. Recently, Mach et al. have reported CPL207280 which, in their in vitro studies, was more effective than TAK-875 [37]. These examples highlight the varying signalling characteristics of different FFA1 activators and the pathways with which the receptor can engage. Whether the optimal characteristics of ligands targeting FFA1 that may have beneficial effects on diabetic patients can be defined and incorporated into ligands that lack the limitations of TAK-875 in terms of bile acid transporter function remain to be ascertained.

**Table 1. Summary of synthetic ligand of long chain free fatty acids pharmacology.**

| Ligand   | Mode of Action                                                                 | Usage            | Status                        | References |
|----------|--------------------------------------------------------------------------------|------------------|-------------------------------|------------|
| TAK-875  | Stimulate intracellular Ca\(^2\+) mobilisation which result in increased blood insulin levels and reduces fasting hyperglycaemia | Type-2 diabetes  | Terminated due to liver toxicity | [45–47]   |
| TUG-770  | Stimulate intracellular Ca\(^2\+) mobilisation which result in increased blood insulin levels | Type-2 diabetes  | Preclinical studies on animals | [40]       |
| TUG-424  | Stimulate intracellular Ca\(^2\+) mobilisation leading to an increase in insulin secretion from pancreatic islets | Type-2 diabetes  | Preclinical studies on animals | [48,49]   |
| TUG-499  | Stimulate intracellular Ca\(^2\+) mobilisation leading to an increase in insulin secretion from pancreatic islets | Type-2 diabetes  | Preclinical studies on animals | [48,50]   |
| TUG-488  | Stimulate intracellular Ca\(^2\+) mobilisation leading to an increase in insulin secretion from pancreatic islets | Type-2 diabetes  | Preclinical studies on animals | [50]       |
| TUG-469  | Stimulate intracellular Ca\(^2\+) mobilisation which significantly improved glucose tolerance | Type-2 diabetes  | Preclinical studies on animals | [51]       |
| Ligand      | Mode of Action                                                                 | Usage             | Status                           | References |
|-------------|--------------------------------------------------------------------------------|-------------------|---------------------------------|------------|
| DS-1558     | Stimulate intracellular Ca\(^{2+}\) mobilisation which significantly improved glucose tolerance insulin secretion from pancreatic islets | Type-2 diabetes   | Preclinical studies on animals   | [41]       |
| GW9508      | Stimulate intracellular Ca\(^{2+}\) mobilisation leading to stimulation of insulin release from pancreatic islets | Type-2 diabetes   | Preclinical studies on animals   | [52]       |
| LY2881835   | Stimulate intracellular Ca\(^{2+}\) mobilisation Immediate and prolonged increase of GLP-1 secretion | Type-2 diabetes   | Stopped after phase I trial      | [53–55]   |
| AM-1638     | Stimulate intracellular Ca\(^{2+}\) mobilisation leading to stimulation of insulin release from pancreatic islets and an increase in GLP-1 secretion from the gut | Preclinical studies on animals | [49,56–58] |
| AM-5262     | Stimulate intracellular Ca\(^{2+}\) mobilisation leading to stimulation of insulin release from pancreatic islets and an increase in GLP-1 secretion from the gut | Preclinical studies on animals | [49,56–58] |
| FFA1 antagonists |                                                                        |                   |                                 |            |
| GW1100      | Inhibiting cell motility                                                         | Osteosarcoma, pituitary cultures, colon cancer cells and enteroendocrine L-cells | Preclinical studies on animals | [59–62]   |
| ANT203      | Inhibition of FFA1 receptor function during the long-term exposure to palmitate resulting in low fatty acid oxidation and positive effects on pancreatic β-cell function | Protects against β-cell dysfunction and apoptosis induced by chronic FFA exposure | Preclinical studies on animals | [63]       |
| DC260126    | Improve insulin sensitivity and protect pancreatic β-cells from apoptosis in diabetic mice | Treatment of type-2 diabetes | Preclinical studies on animals | [64,65]   |
| FFA4 agonists                             |                                                                        |                   |                                 |            |
| GW9508      | Blocking extracellular signal-regulated kinase activity                          | Prevent fasting-induced plasma ghrelin elevation | Preclinical studies on animals | [66]       |
| NCG21       | Activates extracellular signal-regulated kinase Stimulate intracellular Ca\(^{2+}\) level | Increase plasma GLP-1 levels | Preclinical studies on animals | [67]       |
| TUG-891     | Stimulate intracellular Ca\(^{2+}\) mobilisation β-arrestin recruitment Activates extracellular signal-regulated kinase | Preclinical studies on animals | [68–70] |
Table 1. Cont.

| Ligand         | Mode of Action                                                                 | Usage                                      | Status                        | References     |
|----------------|--------------------------------------------------------------------------------|--------------------------------------------|-------------------------------|----------------|
| TUG-1197       | Stimulate intracellular Ca^{2+} level β-arrestin recruitment                      | Type-2 diabetes                           | Preclinical studies on animals | [71]           |
| GSK137647A     | Suppress the adipogenic differentiation of bone mesenchymal stem cells          | Osteogenic and adipogenic differentiation of bone mesenchymal stem cells Type-2 diabetes | Preclinical studies on animals | [72,73]       |
| Merck Compound A | β-arrestin recruitment Stimulate intracellular Ca^{2+} mobilisation              | Anti-inflammatory effects in macrophages Type-2 diabetes | Preclinical studies on animals | [14,74]       |
| Metabolex 36   | Activation of Gαi/o                                                             | Study FFA4 function in pancreatic δ cells  | Preclinical studies on animals | [75]           |
| Metabolex B    | Activation of Gαi/o                                                             | Role of FFA4 in ghrelin secretion and somatostatin release from mouse pancreas | Preclinical studies on animals | [76,77]       |
| KDT501         | Help in control ling impaired glucose tolerance and insulin sensitivity in patient with insulin resistance | Type-2 diabetes                           | Preclinical studies on animals | [78]           |
| AH7614         | Inhibit the intracellular release of Ca^{2+}                                    | Type-2 diabetes                           | Preclinical studies on animals | [79–81]       |

2. Free Fatty Acid Receptor 4

Additionally, known as GPR120, FFA4 was de-orphanised as an FFA receptor almost 20 years ago [4,18,82,83]. It responds to a similar group of long chain FFAs as FFA1, and is also a member of the rhodopsin subgroup of GPCRs [83]. FFA4 has been identified in different tissues and may serve different physiological roles [84,85] (Figure 1). FFA4 is expressed abundantly in the intestinal tract [18,86] and Lu et al. (2021) and Hirasawa and co-workers (2005) demonstrated that the stimulation of FFA4 promotes release of glucagon-like peptide-1 (GLP-1) from gut cells [18,87]. However, the importance of this receptor to this end-point remains contentious [8,14,85,87,88]. In the duodenum, FFA4 shows high co-localisation with the satiety hormone ghrelin [89]. FFA4 is also expressed in other specific cell types, such as K cells and brush cells. In K cells it promotes the release of insulin [87] whilst in brush cells, FFA4 was found at the limited ridge of the mouse stomach, making FFA4 a reasonable candidate to sense long chain fatty acids in the lumen [90]. Expression of FFA4 in other tissues, including lung, heart and skeletal muscle, is known but the physiological roles of FFA4 in these tissues need further study before they can be fully understood. However, in recent years its roles in the lung [13] and in δ cells of the pancreas [14] have been investigated. In addition, FFA4 knockout mice develop obesity when fed a high-fat diet and glucose intolerance and fatty livers were noted in such animals lacking FFA4 [91]. Such observations have increased the motivation to study FFA4, often in the context of diabetes and other metabolic syndromes [71]. In the treatment of type-2 diabetes, FFA4 ligands have attracted considerable interest because of their ability to enhance glucose-dependent insulin production from pancreatic β-cells as well as their anti-inflammatory actions in adipocytes and the gut [85] (Table 1). Stimulation
of this receptor by oleic acid enhances lipid droplet formation in adipose tissues via the G_q, PI3K-Akt, and PLC signalling pathways, while in enteroeendocrine cells it regulates hormone production by promoting GLP-1 release and inhibiting ghrelin secretion [18,92,93]. Although not restricted to polyunsaturated fatty acids (PUFAs), there has been interest in whether such ligands, especially those of the ω-3 family, may regulate a distinct subset of the capabilities of the FFA4 receptor [73,93–95]. The development of synthetic FFA4 selective agonists has also been central to gaining a deeper knowledge of the biological actions of this receptor. Many of the initially described FFA4 active ligands were closely related to fatty acids, and ligands such as GW9508 and NCG21, show only a modest selectivity for FFA4 over FFA1 [65,96,97]. The first potent and selective FFA4 agonist was TUG-891 [98] and this is still by far the most widely used ligand in the literature. Moreover, the phenylpropionic acid core of this molecule has been the inspiration for a wide range of subsequently reported FFA4 active and selective agonists [4,8]. Despite being a potent and selective FFA4-selective ligand in humans, TUG-891 displays a lower selectivity between the mouse orthologues of FFA4 and FFA1 [99], and this needs to be considered when using mouse models of disease. Other FFA4 ligands that have been used relatively widely in the literature include GSK137647A [72], Merck Compound A [14], Metabolex 36 and Metabolex compound B [77], and TUG-1197 [71].

Ligands acting on FFA4 promote receptor interactions with G_q/11 and hence activate phospholipase C, and boost Ca^{2+} levels (Figure 3), which ultimately results in the release of L cell-expressed peptide hormones [100]. As with FFA1, that selective G_q/G_11 blockers, such as FR900359 and YM-254890 are able to ablate such signals, demonstrates the significance of this G protein subfamily in the fundamental components of FFA4 actions [99]. Moreover, this is supported by the fact that FFA4 lacked the ability to increase inositol phosphates or intracellular Ca^{2+} concentrations when expressed in HEK293 cells in which the expression of both G_q and G_11 that had been eliminated by genome editing [101]. This signalling route is fundamental to many of the effects that FFA4 has in physiological settings [8,82,101,102]. On the other hand, treatment with pertussis toxin, which defines a function for G_i-family G proteins, eliminates FFA4-mediated production of the hormone ghrelin [76]. It is clear that agonist-activated FFA4 interacts rapidly, and in a sustained fashion, with β-arrestins and this results in the rapid desensitisation of G protein-mediated functions and in receptor internalisation [99]. These observations pose questions about the potential challenges in targeting FFA4 via therapeutically [100] that remain to be fully understood and resolved. Although a large body of data indicates that the activation of FFA4 can have profound anti-inflammatory effects the basis of these remains uncertain. This is despite effects in macrophages clearly appearing to involve a cascade initiated by the β-arrestin-mediated scaffolding of adaptor proteins [74,94]. The importance of arrestin-mediated signalling may indeed be cell type-dependent. This has been seen by Alvarez-Curto et al. [101] who failed be a contribution of arrestins to FFA4-mediated stimulation of ERK1/2 phosphorylation by utilising HEK293 cells that had been genome-edited to lack expression of the two β-arrestins. This was despite parental HEK293 cells often being used to implicate ERK1/2 phosphorylation as an arrestin-mediated outcome of the activation of heterologously expressed GPCRs [101]. By contrast, in such arrestin-null cells the desensitisation of G protein-mediated FFA4 signalling was abrogated, implying that the more traditional ‘arresting’ role of the β-arrestins had indeed been eliminated [101].

Although no synthetic FFA4 selective agonist has yet entered clinical trials in any disease setting [102], there have nevertheless been small scale clinical studies conducted using naturally occurring FFA4 active ligands. For example, healthy and obese patients were given pine nut oil and olive oil to consume in order to investigate the impact of these natural oils on glucose tolerance and on both FFA1, FFA4 and a further ‘metabolic’ receptor GPR119 [103,104]. Pinoletic acid, the major fatty acid component of pine nut oil is an equipotent, natural dual agonist of FFA1 and FFA4 and enhances glucose tolerance in rodents [105].
Figure 3. FFA4 engages with a variety of pathways to control signaling and physiological functions. Ligand-induced interactions of FFA4 with $G_q/G_{11}$ G proteins leads to increased intracellular $[Ca^{2+}]$ levels. This pathway is fundamental to many of the effects that FFA4 has in physiological settings. Numerous efforts to develop synthetic ligands of FFA4 have employed receptor-$\beta$-arrestin interaction assays. A key physiological function of FFA4 engagement with a $\beta$-arrestin is the production of anti-inflammatory mediators by macrophages. A number of studies have defined the key sites of agonist-mediated, GRK-dependent (here shown as GRK6) phosphorylation in both human and mouse FFA4 (see text). A transgenic knock-in mouse line expressing a phosphorylation-deficient (PD mouse) form of FFA4 is available [82,96] and this will help to assess the specific roles and functions of phosphorylation of FFA4, including in mouse models of disease. Studies have also shown an important role for FFA4 interactions with pertussis-toxin-sensitive G proteins to control the release of the satiety hormone ghrelin [76].

2.1. The GRK Family

GRKs are a class of 7 serine/threonine protein kinases that are most closely linked to the AGC kinase family. On the basis of their sequence similarities, members of the GRK family are categorised into the following three subgroups: the rhodopsin kinase or visual GRK subgroup comprising GRK1 and GRK7, the ‘$\beta$-adrenergic’ receptor kinase subgroup of GRK2 and GRK3, and the GRK4 subgroup which is comprised of GRK4, GRK5 and GRK6 [106]. Although similar in terms of targeting GPCRs, the subgroups have their own distinctive regulatory features. The expression of GRK2, 3, 5, and 6 is widespread across mammalian tissues, but expression of GRK1, 4, and 7 is limited to certain organs [107,108]. GRK4 is found in the testes, cerebellum, and kidneys [109,110] whilst GRK1 and 7 are found predominantly in the rods and cones of the retina, respectively [109]. Modularity is achieved in GRKs by the presence of a short amino-acid terminal a-helical domain (N-helix) and a variable carboxy terminal lipid-binding region [111]. The catalytic region of GRKs is located inside the regulator of the G protein trafficking homology (RH) region [112]. The regulated phosphorylation of the vast majority of GPCRs is under the stringent control of the four ubiquitously expressed GRKs [112], although the contribution of each may be receptor and cell type specific. Desensitisation, internalisation, and their functional consequences are the results of the engagement of GRKs with particular receptors. Subsequent de-phosphorylation is important to allow receptors to recycle back to the cell surface [113,114]. Variation allows diversity, with some GPCRs showing sustained intracellular trafficking, which localises the receptors to particular intracellular compartments, and this may potentially lead to a second wave of endosomal-generated signalling. Such
localisation of the receptors is achieved through the GPCRs’ ability to bind to their ligands for longer periods of time [115]. Within a cellular setting, the act of GRK-binding causes active GPCRs to undergo intracellular activation at the places where they are located.

GRKs play not only a vital role as regulators but also determine the actions of β-arrestins by causing ligand-specific GPCR activation or by preferentially coupling to particular active receptor regions [107]. The field of structural biology has made significant contributions to our comprehension of the architectural changes that occur in receptors before they engage in contact with G proteins or arrestins. The ubiquitous expression of GRK2, 3, 5, and 6 has made it difficult to understand the functions that each particular GRK plays in the process of receptor activation [116]. However, the use of selective small molecule GRK inhibitors, such as compound 101 to block GRK 2/3 [117] and compound 18 to block GRK5/6 [118], siRNA/shRNA methods [116,119] or CRISPR/Cas9 approaches targeting only a specific subset of relevant GRKs [107,120], is beginning to unravel the mysteries of these topics. In addition, the use of phospho-site-specific antibodies [16,121,122] and mass spectrometry analysis of the sites of regulated phosphorylation [16,96,122,123] are also providing valuable insights. For example, Marsango et al. (2022) identified a crucial pair of threonine residues in the medium chain fatty acid receptor GPR84 that are only phosphorylated in response to receptor activation, and this occurs, at least in HEK293 cells, via GRK2/3 [16]. This regulation defines efficient interactions with arrestins and allows the separation of G protein-biased and more balanced GPR84 agonists [16]. Similarly, Divorty et al. (2022) reported phospho-site-specific antisera that act as activation state-specific biomarkers for the orphan metabolite receptor GPR35 [122]. Here, pre-treatment with the GRK2/3 blocker compound 101 significantly decreased the agonist-induced phosphorylation of human, and particularly mouse, orthologues of GPR35, as detected by these antisera. These studies indicate a critical function for GRK2 and/or GRK3 on key residues to control interactions with arrestins [122].

It is not known if a particular GPCR is activated by one GRK or by numerous GRKs in a sequential and potentially hierarchical manner. Drube et al. (2022) were able to show that various GRKs and second messenger kinases are able to induce diverse outcomes based on the targeted GPCR [107]. For example, the activity of GRKs can be either increased or decreased via the action of Protein Kinase C (PKC) and the presence of Gq-family G proteins. They recorded a decrease in GRK5- and GRK6-mediated β-arrestin recruitment to the angiotensin AT1R when PKC activity was suppressed. Clearly, however, much further analysis will still be required.

GRK2 and GRK3 are typically found within the cytosol in the absence of GPCR stimulation, but they are nevertheless able to translocate to the cell surface upon stimulation due to their engagement with the βγ-heterodimer of active G proteins [124]. By contrast, GRK5 and GRK6 are routinely membrane-localised [106]. It is possible, particularly in more complex native cells, that some receptors are found in membrane regions that are inaccessible to GRK5 and GRK6, and this may in part help shape the effects of different GRKs.

2.2. Arrestin 2 and Arrestin 3

There are four members of the arrestin family, namely, arrestin-2 (also known as β-arrestin 1 and arrestin-3 (β-arrestin 2), which share 78% sequence identity [125], and are widely distributed within the body. Both β-arrestin 1 and β-arrestin 2 control a variety of signalling pathways by interacting with the majority of non-visual GPCRs [112]. The potential of the β-arrestins in regulating and mediating various functions of GPCRs is well-established but the exact roles of other arrestin-like molecules, including arrestin domain-containing proteins and α-arrestins, in receptor endocytosis and other functions are not yet fully understood [126,127]. Initially β-arrestins were thought simply to function as down-regulators of GPCR signalling by preventing and ‘arresting’ GPCR g protein interactions. However, over time, a much broader set of roles in providing scaffolds for distinct signalling pathways has been uncovered [128,129]. These include the scaffolding of proteins that have a role in signal transduction pathways, including members of mitogen-
activated protein kinase cascades, E3 ubiquitin ligases and Src family tyrosine kinases [129].

In terms of specificity, β-arrestin 2, but not β-arrestin 1, was reported to scaffold the ASK1-MKK4/7-JNK3 cascade in a receptor-dependent manner [112,130]. Recent research has suggested an important interplay between arrestin-stimulated signalling and GPCR engagement as it has been indicated that arrestin functioning beyond roles in desensitisation may be lacking in the absence of G protein activity [101,131].

2.3. Roles of GRKs and Arrestins in the Functions of FFA1 and FFA4

As described earlier, GRKs and arrestins are key adapter and scaffold molecules that can mediate the actions of a variety of distinct GPCRs [112] and, not surprisingly, both FFA1 and FFA4 have been studied in this regard. An early study was unable to record FFA1-induced β-arrestin recruitment to FFA1 [132], potentially suggesting a limited role for this in the signal transduction of FFA1. However, Qian et al. (2014) demonstrated that both β-arrestin 2 and GRK2 play roles in the linoleate-induced, clathrin-mediated internalisation of FFA1 from the surface of transfected HEK293 cells, whereas constitutive internalisation of the receptor did not utilise this route [35]. Perhaps more directly relevant to pancreatic function, knock-down or genetic elimination of β-arrestin 2 in an insulin-secreting cell line and in mouse islets, respectively, limited the insulinotropic activity of the FFA1 agonist TAK-875 [34]. Despite these studies, there has so far only been limited analysis of ligand regulated phosphorylation of FFA1. Recently Guzmán-Silva et al. (2022) performed mutagenesis on a number of serine and threonine residues in the 3rd intracellular loop and C-terminal tail of human FFA1 and observed that, although not affecting the docosahexaenoic acid (DHA)-induced elevation of Ca$^{2+}$ when the mutants were expressed in HEK293 cells, these mutants were substantially less well phosphorylated in response to DHA compared to the wild type receptor [133]. Moreover, a mutant with alterations in both the C-terminal tail and 3rd intracellular loop was internalised very poorly [133]. Perhaps surprisingly, the characterisation of phospho-site specific antisera for FFA1 or the role of specific GRKs have not yet been reported. This is certainly not the case for FFA4: at this receptor Burns et al. (2014) used a knockdown strategy to indicate that, at least again in HEK293 cells, GRK6 was largely responsible for DHA-mediated phosphorylation of the receptor and that residues Thr$^{347}$, Ser$^{350}$ and Ser$^{357}$ in the C-terminal tail were likely sites of modification [134]. These studies were rapidly complemented by Butcher et al. [135]. Using combinations of mass spectrometry, mutagenesis, and the development of phospho-site specific antisera, they showed that each of Thr$^{347}$, Thr$^{349}$, Ser$^{350}$, Ser$^{357}$, and Ser$^{360}$ in the C-terminal tail of human FFA4 became phosphorylated in response to the synthetic FFA4 agonist TUG-891 when the receptor was expressed in either CHO or HEK293 cells. Although the roles of specific GRKs were not examined, the use of a pSer$^{347}$-pThr$^{350}$ site-specific antiserum showed that TUG-891-induced phosphorylation of these residues was not mediated via PKC and hence probably reflected the actions of one or more GRKs [135]. Truncation from the C-terminus to delete sites of agonist-mediated phosphorylation progressively prevented TUG-891 induced recruitment of β-arrestin 2 and receptor internalisation. Moreover, mutation to alanine of all the identified sites of agonist-induced phosphorylation restricted, but did not fully eliminate, interactions with β-arrestin 2. To do so required further substitution of acidic residues in the C-terminal tail. Replacement by alanine of no single hydroxy-amino acid was sufficient to produce a substantial effect on TUG-891-induced β-arrestin 2 recruitment, indicating the need for phosphorylation of a number of distinct residues [135]. Subsequently Prihandoko et al. explored similar themes for the mouse orthologue of FFA4 [96]. As well as showing agonist-induced phosphorylation of this orthologue at equivalent residues as in the human orthologue, these studies went on to show that mutation of only the phospho-acceptor sites at the extreme C-terminus was sufficient to limit receptor internalisation and β-arrestin 2 recruitment [96]. In contrast mutation of the more proximal residues to alanines had a limited effect on β-arrestin 2 recruitment but a substantial effect on the downstream activation of pAkt [96]. This interesting observation raises the potential for FFA4 active
agonists to act in a ‘biased’ signalling fashion if they are able to phosphorylate selectively these two separable groups of Ser/Thr residues.

This concept, first described by Tobin as the ‘bar-code’ hypothesis [136], suggested that ligands, either due to their inherent nature or to variations in the make-up and expression pattern of cellular kinases and downstream effectors in different cells, could differentially regulate signalling outcomes in different cells and tissues by promoting different phosphorylation ‘bar-codes’ on the intracellular face of GPCRs. This might result in the recruitment of distinct adaptor complexes being favoured by unique phosphorylation patterns. To fully explore this hypothesis for FFA1 and/or FFA4 will require a number of steps. Firstly, the identification and characterisation of chemically distinct activating ligands that display ‘bias’ in induced phosphorylation. For FFA4, as described earlier, to date, many of the agonist ligands are based on the phenylpropionic acid core of TUG-891 and hence there is a lack of chemical diversity. Thus, although sites of phosphorylation, induced by such ligands, are now well described (see above), it is most likely that this will be replicated by other synthetic agonist ligands. It may be of considerable help, however, to explore this for fatty acid ligands of different chain length and extent of unsaturation [105]. By contrast, with both described and well characterized orthosteric and allosteric ligands that bind to at least two distinct non-orthosteric sites, it may be that ligands that display bias in FFA1 phosphorylation bar-coding could already exist, even if they have not yet been assessed in this manner. To progress further with this concept, it will, however, require either more widespread and routine application of mass spectrometry to analyse rapidly potential differences in the sites of ligand-induced phosphorylation in FFA1 and FFA4 in a co-ordinated workflow and/or the production and use of a more robust and complete set of ligand-regulated phospho-site specific antisera that cover the full panoply of such sites in FFA4 and in FFA1. These are not yet available for either receptor. Recent times, however, have seen examples of ligand bar-coding for other GPCRs such as the δ-opioid receptor (DOP) where Mann et al. (2020) used phosphosite-specific antibodies at Thr$^{361}$ and Ser$^{363}$ residues and identified a distinct and hierarchical agonist-induced phosphorylation pattern in the C-terminal tail of DOP [116]. This enabled a definition of a phosphorylation pattern that was aligned with receptor internalisation. Such analysis may support distinct functional effects of DOP ligands within the range of the different functions and effects of this receptor. Further application of such patterns of phosphorylation may allow the identification and characterisation of selective ligands, potentially, those best suited for specific therapeutic applications, and this may then be extended to other GPCRs.

A further potential complication for analysis of FFA4 is that there is an additional FFA4 splice variant in humans [6]. The longer isoform contains an insert of 16 amino acids within the third intracellular loop and this form has been reported to show an intrinsic β-arrestin-bias. Senatorov et al. (2020) recently explored the ability of DHA to promote the phosphorylation of this variant and showed a difference in basal phosphorylation compared to the wild type. Once again however, at least in the background of HEK293 cells, knock-down studies highlighted the important role of GRK6 [137].

Of greater and longer-term importance, all of the studies on the specific nature of GRK subtype regulation of FFA1 and FFA4 have, to date, been performed after heterologous expression of the receptors into simple cell systems. Knock-down/knock-out of GRK isoforms, arrestins and even G proteins, as well as the use of various target and pathway inhibitors, have then allowed analysis of the contribution of specific GRKs and arrestins to the phosphorylation, regulation and signal transduction of a number of GPCRs including FFA1 and FFA4. Ultimately such studies can only hint at the specific roles for these proteins in the regulation of their function in vivo. A start has already been made to explore this through the generation of a transgenic mouse-line, in which FFA4 was replaced by a phospho-deficient (PD) form (Figure 4). Analysis of this line, as well as of a range of other similar transgenic lines that could be produced, will start to provide the levels of insight required to gain a fuller understanding and, hopefully, successful therapeutic programmes targeting these receptors.
Figure 4. Production and use of a transgenic knock-in FFA4 phosphorylation deficient mouse line. After treatment of with an agonist ligand, FFA4 receptors undergo rapid phosphorylation on two sets of Ser/Thr sites located within the intracellular C-tail of the receptor. To explore the role of regulated phosphorylation of FFA4, the wild type receptor was replaced with a variant in which all the hydroxy-amino acids in the C-terminal tail were replaced by alanines (phospho-deficient, PD). In addition, to facilitate detection of the expressed receptor protein, the HA-epitope tag sequence was placed in-frame at the C-terminus, as previously reported for the free fatty acid receptor 2 [138–140]. Together with the wild type and FFA4 knock-out littermates, a comprehensive understanding of the role of the phosphorylation of FFA4 may be established by studying this line.

Author Contributions: A.G.A., A.B.T. and G.M. wrote the article. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by [Medical Research Council] grant number [MR/R00305X/1] to G.M. and A.T.B.

Acknowledgments: Abdulrahman G. Alharbi is supported by a research scholarship from Taibah University—Ministry of Education—Kingdom of Saudi Arabia.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Berry, S.E.; Bruce, J.H.; Steenson, S.; Stanner, S.; Buttriss, J.L.; Spiro, A.; Gibson, P.S.; Bowler, I.; Dionisi, F.; Farrell, L.; et al. Interesterified Fats: What Are They and Why Are They Used? A Briefing Report from the Roundtable on Interesterified Fats in Foods. Nutr. Bull. 2019, 44, 363–380. [CrossRef]
2. Shahidi, F.; Ambigaipalan, P. Omega-3 Polyunsaturated Fatty Acids and Their Health Benefits. Annu. Rev. Food Sci. Technol. 2018, 9, 345–381. [CrossRef]
3. Blad, C.C.; Tang, C.; Offermanns, S. G Protein-Coupled Receptors for Energy Metabolites as New Therapeutic Targets. Nat. Rev. Drug Discov. 2012, 11, 603–619. [CrossRef] [PubMed]
4. Milligan, G.; Shimpukade, B.; Ulven, T.; Hudson, B.D. Complex Pharmacology of Free Fatty Acid Receptors. Chem. Rev. 2017, 117, 67–110. [CrossRef] [PubMed]
5. Hauser, A.S.; Attwood, M.M.; Rask-Andersen, M.; Schiöth, H.B.; Gloriam, D.E. Trends in GPCR Drug Discovery: New Agents, Targets and Indications. Nat. Rev. Drug Discov. 2017, 16, 829–842. [CrossRef]
6. Holliday, N.D.; Watson, S.J.; Brown, A.J.H. Drug Discovery Opportunities and Challenges at G Protein Coupled Receptors for Long Chain Free Fatty Acids. Front. Endocrinol. 2012, 3, 112. [CrossRef]
7. Sharma, N.; Bhagat, S.; Chundawat, S.T. Recent Advances in Development of GPR40 Modulators (FFA1/FFAR1): An Emerging Target for Type 2 Diabetes. Mini Rev. Med. Chem. 2017, 17, 947–958. [CrossRef]
8. Carullo, G.; Mazzotta, S.; Vega-Holm, M.; Iglesias-Guerra, F.; Vega-Perez, J.M.; Aiello, F.; Brizzi, A. GPR120/FFAR4 Pharmacology: Focus on Agonists in Type 2 Diabetes Mellitus Drug Discovery. *J. Med. Chem.* 2021, 64, 4312–4332. [CrossRef]

9. Grundmann, M.; Bender, E.; Schamberger, J.; Eitner, F. Pharmacology of Free Fatty Acid Receptors and Their Allosteric Modulators. *Int. J. Mol. Sci.* 2021, 22, 1763. [CrossRef]

10. Tikhonova, I.G.; Poerio, E. Free Fatty Acid Receptors: Structural Models and Elucidation of Ligand Binding Interactions. *BMC Struct. Biol.* 2015, 15, 16. [CrossRef]

11. Alvarez-Curto, E.; Milligan, G. Metabolism Meets Immunity: The Role of Free Fatty Acid Receptors in the Immune System. *Biochem. Pharmacol.* 2016, 114, 3–13. [CrossRef]

12. Kimura, I.; Ichimura, A.; Ohtake-Kitano, R.; Igarashi, M. Free Fatty Acid Receptors in Health and Disease. *Physiol. Rev.* 2020, 100, 171–210. [CrossRef] [PubMed]

13. Prihandoko, R.; Kaur, D.; Wiegman, C.H.; Alvarez-Curto, E.; Donovan, C.; Chachi, L.; Ulven, T.; Euston, E.; Dong, Z.; et al. Pathophysiological Regulation of Lung Function by the Free Fatty Acid Receptor FFA4. *Sci. Transl. Med.* 2020, 12, eaaw9009. [CrossRef]

14. Croze, M.L.; Filshner, M.; Guillaume, A.; Tremblay, C.; Noguchi, G.M.; Granzierza, S.; Vivot, K.; Castillo, V.C.; Campbell, S.A.; Ghislain, J.; et al. Free Fatty Acid Receptor 4 Inhibitory Signaling in Delta Cells Regulates Islet Hormone Secretion in Mice. *Mol. Metab.* 2021, 45, 101166. [CrossRef]

15. Patti, A.M.; Giglio, R.V.; Papanas, N.; Serban, D.; Stoian, A.P.; Pafili, K.; Al Rasadi, K.; Rajagopalan, K.; Rizvi, A.A.; Ciaccio, M.; et al. Experimental and Emerging Free Fatty Acid Receptor Agonists for the Treatment of Type 2 Diabetes. *Medicina* 2022, 58, 109. [CrossRef]

16. Marsango, S.; Ward, R.J.; Jenkins, L.; Butcher, A.J.; Al Mahmud, Z.; Dwomoh, L.; Nagel, F.; Schulz, S.; Tikhonova, I.G.; Tobin, A.B.; et al. Selective Phosphorylation of Threonine Residues Defines GPR84–Arrestin Interactions of Biased Ligands. *J. Biol. Chem.* 2022, 298, 101932. [CrossRef]

17. Hara, T.; Kimura, I.; Inoue, D.; Ichimura, A.; Hirasawa, A. Free Fatty Acid Receptors and Their Role in Regulation of Energy Metabolism. In *Reviews of Physiology, Biochemistry and Pharmacology*; Nilius, B., Amara, S.G., Lill, R., Offermanns, S., Gudermann, T., Petersen, O.H., Jahn, R., Eds.; Springer International Publishing: Cham, Switzerland, 2013; Volume 164, pp. 77–116. [CrossRef]

18. Hirasawa, A.; Tsumura, K.; Awaib, T.; Katsuma, S.; Adachi, T.; Yamada, M.; Sugimoto, Y.; Miyazaki, S.; Tsujimoto, G. Free Fatty Acids Regulate Gut Incretin Glucagon-like Peptide-1 Secretion through GPR120. *Nat. Med.* 2005, 11, 90–94. [CrossRef]

19. Briscoe, C.P.; Tadayyon, M.; Andrews, J.L.; Benson, W.G.; Chambers, J.K.; Eilert, M.M.; Ellis, C.; Elshourbagy, N.A.; Goetz, A.S.; Hara, T.; Kimura, I.; Inoue, D.; Ichimura, A.; Hirasawa, A. Free Fatty Acid Receptors and Their Role in Regulation of Energy Metabolism. In *Reviews of Physiology, Biochemistry and Pharmacology*; Nilius, B., Amara, S.G., Lill, R., Offermanns, S., Gudermann, T., Petersen, O.H., Jahn, R., Eds.; Springer International Publishing: Cham, Switzerland, 2013; Volume 164, pp. 77–116. [CrossRef]

20. Itoh, Y.; Kawamata, Y.; Harada, M.; Kobayashi, M.; Fujii, R.; Fukusumi, S.; Ogi, K.; Hosoya, M.; Tanaka, Y.; Uejima, H.; et al. Free Fatty Acids Regulate Insulin Secretion from Pancreatic β Cells through GPR40. *Sci. Transl. Med.* 2013, 5, 184ra25. [CrossRef] [PubMed]

21. Takasaki, J.; Saito, T.; Taniguchi, M.; Kawasaki, T.; Moritani, Y.; Hayashi, K.; Kobori, M. A Novel Gαq/11-Selective Inhibitor. *J. Biol. Chem.* 2004, 279, 47438–47445. [CrossRef]

22. Latour, M.G.; Alquier, T.; Oseid, E.; Tremblay, C.; Jetton, T.L.; Luo, J.; Lin, D.C.H.; Poitout, V. GPR40 Is Necessary but Not Sufficient for Fatty Acid Stimulation of Insulin Secretion in Vivo. *Diabetes* 2007, 56, 1087–1094. [CrossRef]

23. Schrage, R.; Schmitz, A.L.; Gaffal, E.; Annala, S.; Kehraus, S.; Wenzel, D.; Büllesbach, K.M.; Bald, T.; Inoue, A.; Shinjo, Y.; et al. The Experimental Power of FR900359 to Study Gq-Regulated Biological Processes. *Nat. Commun.* 2015, 6, 10156. [CrossRef]

24. Dang, H.; Krause, L.; Dong, Z.; et al. Novel G Protein-Coupled Receptor GPR40 Is Activated by Medium and Long Chain Fatty Acids. *Biochem. Pharmacol.* 2013, 85, 91–100. [CrossRef]

25. Milligan, G.; Ulven, T.; Murdoch, H.; Hudson, B.D. G-Protein-Coupled Receptors for Free Fatty Acids: Nutritional and Therapeutic Targets. *Br. J. Nutr.* 2014, 111, S3–S7. [CrossRef]

26. Ghislain, J.; Poitout, V. Targeting LipidGPCRs to Treat Type 2 Diabetes Mellitus—Progress and Challenges. *Nat. Rev. Endocrinol.* 2021, 17, 162–175. [CrossRef]

27. Tan, C.P.; Feng, Y.; Zhou, Y.-P.; Eiermann, G.J.; Petrov, A.; Zhou, C.; Lin, S.; Salituro, G.; Meinke, P.; Mosley, R.; et al. Selective Small-Molecule Agonists of G Protein-Coupled Receptor 40 Promote Glucose-Dependent Insulin Secretion and Reduce Blood Glucose in Mice. *Diabetes* 2008, 57, 2211–2219. [CrossRef]

28. Lin, D.C.-H.; Guo, Q.; Luo, J.; Zhang, J.; Nguyen, K.; Chen, M.; Tran, T.; Dransfield, P.J.; Brown, S.P.; Houze, J.; et al. Identification and Pharmacological Characterization of Multiple Allosteric Binding Sites on the Free Fatty Acid 1 Receptor. *Mol. Pharmacol.* 2012, 82, 843–859. [CrossRef]

29. Lin, D.C.-H.; Zhang, J.; Zhuang, R.; Li, F.; Nguyen, K.; Chen, M.; Tran, T.; Lopez, E.; Lu, J.Y.L.; Li, X.N.; et al. AMG 837: A Novel GPR40/FFA1 Agonist That Enhances Insulin Secretion and Lowers Glucose Levels in Rodents. *PLoS ONE* 2011, 6, e27270. [CrossRef]

30. Fujiwara, K.; Maekawa, F.; Yada, T. Oleic Acid Interacts with GPR40 to Induce Ca2+ Signaling in Rat Islet β-Cells: Mediation by PLC and L-Type Ca2+ Channel and Link to Insulin Release. *Am. J. Physiol.-Endocrinol. Metab.* 2005, 289, 670–677. [CrossRef]
33. Feng, D.D.; Luo, Z.; Roh, S.; Hernandez, M.; Tawadros, N.; Keating, D.J.; Chen, C. Reduction in Voltage-Gated K+ Currents in Primary Cultured Rat Pancreatic β-Cells by Linoleic Acids. *Endocrinology* **2006**, *147*, 674–682. [CrossRef]

34. Mancini, A.D.; Bertrand, G.; Vivot, K.; Carpenter, É.; Tremblay, C.; Ghislain, J.; Bouvier, M.; Poitout, V. β-Arrestin Recruitment and Biased Agonism At Free Fatty Acid Receptor 1. *J. Biol. Chem.* **2015**, *290*, 21131–21140. [CrossRef]

35. Qian, J.; Wu, C.; Chen, X.; Li, X.; Ying, G.; Jin, L.; Ma, Q.; Li, G.; Shi, Y.; Zhang, G.; et al. Differential Requirements of Arrestin-3 and Clathrin for Ligand-Dependent and -Independent Internalization of Human G Protein-Coupled Receptor 40. *Cell. Signal.* **2014**, *26*, 2412–2423. [CrossRef]

36. Kim, M.; Gu, G.J.; Koh, Y.-S.; Lee, S.-H.; Na, Y.R.; Seok, S.H.; Lim, K.-M. Fasiglifam (TAK-875), a G Protein-Coupled Receptor 40 (GPR40) Agonist, May Induce Hepatotoxicity through Reactive Oxygen Species Generation in a GPR40-Dependent Manner. *Biomol. Ther.* **2018**, *26*, 599–607. [CrossRef]

37. Mach, M.; Bazydło-Guzenda, K.; Buda, P.; Matłoka, M.; Dzida, R.; Stelmach, F.; Gałąźka, K.; Wasińska-Kałwa, M.; Smuga, D.; Holowirska, D.; et al. Discovery and Development of CPL207280 as New GPR40/FFA1 Agonist. *Eur. J. Med. Chem.* **2021**, *226*, 113810. [CrossRef]

38. Suckow, C.P. Key Questions for Translation of FFA Receptors: From Pharmacology to Medicines. In *Free Fatty Acid Receptors*; Milligan, G., Kimura, I., Eds.; Springer International Publishing: Cham, Switzerland, 2016; Volume 236, pp. 101–131. [CrossRef]

39. Li, Z.; Qiu, Q.; Geng, X.; Yang, J.; Huang, W.; Qian, H. Free Fatty Acid Receptor Agonists for the Treatment of Type 2 Diabetes: Drugs in Preclinical to Phase II Clinical Development. *Expert Opin. Investig. Drugs* **2016**, *25*, 871–890. [CrossRef]

40. Christiansen, E.; Hansen, S.V.F.; Urban, C.; Hudson, B.D.; Wargent, E.T.; Grundmann, M.; Jenkins, L.; Zaibi, M.; Stocker, C.J.; Ullrich, S.; et al. Discovery of TUG-770: A Highly Potent Free Fatty Acid Receptor 1 (FFA1/GPR40) Agonist for Treatment of Type 2 Diabetes. *ACS Med. Chem. Lett.* **2013**, *4*, 441–445. [CrossRef]

41. Takano, R.; Yoshida, M.; Inoue, M.; Honda, T.; Nakashima, R.; Matsumoto, K.; Yano, T.; Ogata, T.; Watanabe, N.; Hirouchi, M.; et al. Discovery of DS-1558: A Potent and Orally Bioavailable GPR40 Agonist. *ACS Med. Chem. Lett.* **2015**, *6*, 266–270. [CrossRef]

42. Hauge, M.; Vestmar, M.A.; Husted, A.S.; Ekberg, J.P.; Wright, M.J.; Di Salvo, J.; Weinglass, A.B.; Engelstoft, M.S.; Madsen, A.N.; Lückmann, M.; et al. GPR40 (FFAR1)—Combined Gs and Gq Signaling in Vitro Is Associated with Robust Incretin Secretagogue Action Ex Vivo and in Vivo. *Mol. Metab.* **2014**, *4*, 3–14. [CrossRef]

43. Christiansen, E.; Urban, C.; Merten, N.; Liebscher, K.; Karlsen, K.K.; Hamacher, A.; Spinrath, A.; Bond, A.D.; Drewke, C.; Ullrich, S.; et al. Discovery of Potent and Selective Agonists for the Free Fatty Acid Receptor 1 (FFA1/GPR40) and Potent Free Fatty Acid Receptor 2 (FFA2/G Protein-Coupled Receptor 42). *Eur. J. Med. Chem.* **2021**, *226*, 113810. [CrossRef]

44. Pachanski, M.J.; Kirkland, M.E.; Kosinski, D.T.; Mane, J.; Cheewatrakoolpong, B.; Xue, J.; Szeto, D.; Forrest, G.; Miller, C.; Bunzel, M.; et al. Discovery of Potent and Selective Agonists for the Free Fatty Acid Receptor 1 (FFA(1)/GPR40), a Potential Target for the Treatment of Type 2 Diabetes Mellitus (LY2881835, LY2922083, and LY2922470). *K. A.; et al. The Discovery, Preclinical, and Early Clinical Development of Potent and Selective GPR40 Agonists for the Treatment of Type II Diabetes.* *J. Med. Chem.* **2013**, *56*, 982–992. [CrossRef]

45. Yabuki, C.; Komatsu, H.; Tsujihata, Y.; Maeda, R.; Ito, R.; Matsuda-Nagasumi, K.; Sakuma, K.; Miyawaki, K.; Kikuchi, N.; Takeuchi, H.; et al. A Novel Antidiabetic Drug, Fasiglifam/TAK-875, Acts as an Ago-Allosteric Modulator of FFAR1. *PLoS ONE* **2012**, *7*, e186033. [CrossRef]

46. Christiansen, E.; Hansen, S.V.F.; Urban, C.; Hudson, B.D.; Wargent, E.T.; Grundmann, M.; Jenkins, L.; Zaibi, M.; Stocker, C.J.; Ullrich, S.; et al. Discovery of TUG-469: A Potent and Selective GPR40 Full Agonist Engages the EnteroInsular Axis to Promote Glucose Control in Rodents. *PLoS ONE* **2017**, *12*, e0186033. [CrossRef]

47. Shavadia, J.S.; Sharma, A.; Gu, X.; Neatson, J.; DeLeve, L.; Holmes, D.; Home, P.; Eckel, R.H.; Watkins, P.B.; Granger, C.B. Determination of Fasiglifam-Induced Liver Toxicity: Insights from the Data Monitoring Committee of the Fasiglifam Clinical Trials Program. *Clin. Trials.* **2019**, *16*, 253–262. [CrossRef]

48. Christiansen, E.; Urban, C.; Merten, N.; Liebscher, K.; Karlsen, K.K.; Hamacher, A.; Spinrath, A.; Bond, A.D.; Drewke, C.; Ullrich, S.; et al. Discovery of Potent and Selective Agonists for the Free Fatty Acid Receptor 1 (FFA1/GPR40), a Potential Target for the Treatment of Type II Diabetes. *J. Med. Chem.* **2008**, *51*, 7061–7064. [CrossRef]

49. Luo, J.; Swaminath, G.; Brown, S.P.; Zhang, J.; Guo, Q.; Chen, M.; Nguyen, K.; Tran, T.; Miao, L.; Dransfield, P.J.; et al. A Potent Class of GPR40 Full Agonists Engages the Enterolipid Axial Axis to Promote Glucose Control in Rodents. *PLoS ONE* **2012**, *7*, e46300. [CrossRef]

50. Christiansen, E.; Due-Hansen, M.E.; Urban, C.; Grundmann, M.; Schmidt, J.; Hansen, S.V.F.; Hudson, B.D.; Zaibi, M.; Markussen, S.B.; Hagesaether, E.; et al. Discovery of a Potent and Selective Free Fatty Acid Receptor 1 Agonist with Low Lipophilicity and High Oral Bioavailability. *J. Med. Chem.* **2013**, *56*, 982–992. [CrossRef]

51. Urban, C.; Hamacher, A.; Partke, H.J.; Roden, M.; Schinner, S.; Christiansen, E.; Due-Hansen, M.E.; Ulven, T.; Gohlke, H.; Kassack, M.U. In Vitro and Mouse in Vivo Characterization of the Potent Free Fatty Acid 1 Receptor Agonist TUG-469. *Naunyn. Schmiedebergs. Arch. Pharmacol.* **2013**, *386*, 1021–1030. [CrossRef]

52. Graciano, M.F.; Valle, M.M.; Curi, R.; Carpinelli, A.R. Evidence for the Involvement of GPR40 and NADPH Oxidase in Palmitic Acid-Induced Superoxide Production and Insulin Secretion. *Islets* **2013**, *5*, 139–148. [CrossRef]

53. Hamdouchi, C.; Kahli, S.D.; Patel Lewis, A.; Cardona, G.R.; Zink, R.W.; Chen, K.; Essalou, T.E.; Ficorilli, J.V.; Marcelo, M.C.; Otto, K.A.; et al. The Discovery, Preclinical, and Early Clinical Development of Potent and Selective GPR40 Agonists for the Treatment of Type 2 Diabetes Mellitus (LY2881835, LY2922083, and LY2922470). *J. Med. Chem.* **2016**, *59*, 10891–10916. [CrossRef]

54. Defossa, E.; Wagner, M. Recent Developments in the Discovery of FFA1 Receptor Agonists as Novel Oral Treatment for Type 2 Diabetes Mellitus. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 2991–3000. [CrossRef]
55. A Study of LY2881835 in Healthy People and People with Diabetes. Available online: https://clinicaltrials.gov/ct2/show/NCT01358981 (accessed on 7 September 2022).

56. Potteout, V.; Lin, D.C.-H. Modulating GPR40: Therapeutic Promise and Potential in Diabetes. Drug Discov. Today 2013, 18, 1301–1308. [CrossRef]

57. Brown, S.P.; Dransfield, P.J.; Vinolotatana, M.; Jiao, X.; Zhu, L.; Pattaropong, V.; Sun, Y.; Liu, J.; Luo, J.; Zhang, J.; et al. Discovery of AM-1638: A Potent and Orally Bioavailable GPR40/FFA1 Full Agonist. ACS Med. Chem. Lett. 2012, 3, 726–730. [CrossRef]

58. Wang, Y.; Liu, J.; Dransfield, P.J.; Zhu, L.; Wang, Z.; Du, X.; Jiao, X.; Su, Y.; Li, A.; Brown, S.P.; et al. Discovery and Optimization of Potent GPR40 Full Agonists Containing Tricyclic Spirocycles. ACS Med. Chem. Lett. 2013, 4, 551–555. [CrossRef]

59. Garrel, G.; Simon, V.; Denovelle, C.; Cruciani-Guglielmacci, C.; Migrenne, S.; Counis, R.; Magnan, C.; Cohen-Tannoudji, J. Unsaturated Fatty Acids Stimulate LH Secretion via Novel PKCe and -6 in Gonadotrope Cells and Inhibit GnRH-Induced LH Release. Endocrinology 2011, 152, 3905–3916. [CrossRef]

60. Wu, Q.; Wang, H.; Zhao, X.; Shi, Y.; Jin, M.; Wan, B.; Xu, H.; Cheng, Y.; Ge, H.; Zhang, Y. Identification of G-Protein-Coupled Receptor 120 as a Tumor-Promoting Receptor That Induces Angiogenesis and Migration in Human Colorectal Carcinoma. Oncogene 2013, 32, 5541–5550. [CrossRef]

61. Takahashi, K.; Fukushima, K.; Onishi, Y.; Node, Y.; Inui, K.; Fukushima, N.; Honoki, K.; Tsujiuchi, T. Different Effects of G-Protein-Coupled Receptor 120 (GPR120) and GPR40 on Cell Motile Activity of Highly Migratory Osteosarcoma Cells. Biochem. Biophys. Res. Commun. 2017, 484, 675–680. [CrossRef]

62. Takahashi, K.; Fukushima, K.; Onishi, Y.; Minami, K.; Otagaki, S.; Ishimoto, K.; Fukushima, N.; Honoki, K.; Tsujiuchi, T. Involvement of FFAR1 and FFAR4 in the Regulation of Cellular Functions during Tumor Progression in Colon Cancer Cells. Exp. Cell Res. 2018, 369, 54–60. [CrossRef]

63. Kristinsson, H.; Smith, D.M.; Bergsten, P.; Sargsyan, E. FFAR1 Is Involved in Both the Acute and Chronic Effects of Palmitate on Insulin Secretion. Endocrinology 2013, 154, 4078–4088. [CrossRef]

64. Sun, P.; Wang, T.; Zhou, Y.; Liu, H.; Jiang, H.; Zhu, W.; Wang, H. DC260126: A Small-Molecule Antagonist of GPR40 That Protects against Pancreatic β-Cells Dysfunction in Db/Db Mice. PloS ONE 2013, 8, e66744. [CrossRef]

65. Zhang, X.; Yan, G.; Li, Y.; Zhu, W.; Wang, H. DC260126, a Small-Molecule Antagonist of GPR40, Improves Insulin Tolerance but Not Glucose Tolerance in Obese Zucker Rats. Biomed. Pharmacother. 2010, 64, 647–651. [CrossRef]

66. Gong, Z.; Yoshimura, M.; Aizawa, S.; Kurotani, R.; Zigman, J.M.; Sakai, T.; Sakata, I. G Protein-Coupled Receptor 120 Signaling Regulates Ghrelin Secretion in Vivo and in Vitro. Am. J. Physiol. Metab. 2014, 306, E28–E35. [CrossRef]

67. Suzuki, T.; Igari, S.I.; Hirasewa, A.; Hata, M.; Itoh, Y.; Hirano, T.; Nakagawa, H.; Ogura, M.; et al. Identification of G Protein-Coupled Receptor 120-Selective Agonists Derived from PPARγ Agonists. J. Med. Chem. 2008, 51, 7640–7644. [CrossRef]

68. Gozal, D.; Qiao, Z.; Almendros, I.; Zheng, J.; Khalyfa, A.; Shimpukade, B.; Ulven, T. Treatment with TUG891, a Free Fatty Acid Receptor 4 Agonist, Restores Adipose Tissue Metabolic Dysfunction Following Chronic Sleep Fragmentation in Mice. Int. J. Obes. 2016, 40, 1143–1149. [CrossRef]

69. Schilperoort, M.; Dam, A.D.; Hoeke, G.; Shabalina, I.G.; Okolo, A.; Hanyaloglu, A.C.; Dib, L.H.; Mol, I.M.; Caengprasath, N.; Christiansen, E.; Milligan, G.; et al. Non-Acidic Free Fatty Acid Receptor 4 Agonists with Antidiabetic Activity. Mol. Metab. 2016, 5, 675–680. [CrossRef] [PubMed]

70. Gao, B.; Huang, Q.; Jie, Q.; Lu, W.G.; Wang, L.; Li, X.J.; Sun, Z.; Hu, Y.Q.; Chen, L.; Liu, B.H.; et al. GPR120: A Bi-Potential Mediator to Modulate the Osteogenic and Adipogenic Differentiation of BMSCs. Sci. Rep. 2015, 5, 14080. [CrossRef]

71. Azevedo, C.M.G.; Watterson, K.R.; Wargent, E.T.; Hansen, S.V.F.; Hudson, B.D.; Kępczyńska, M.A.; Dunlop, J.; Shimpukade, B.; Ulven, T. Treatment with TUG891, a Free Fatty Acid Receptor 4 Agonist, Restores Adipose Tissue Metabolic Dysfunction Following Chronic Sleep Fragmentation in Mice. Int. J. Obes. 2016, 40, 1143–1149. [CrossRef]

72. Wang, C.; Liu, Y.; Pan, Y.; Jin, H. Effect of GSK-376474A, the First Non-Carboxylic FFA4 Agonist, on the Osteogenic and Adipogenic Differentiation of Bone Mesenchymal Stem Cells in Db/Db Mice. J. Pharm. Pharmacol. 2020, 72, 461–469. [CrossRef]

73. Wang, Y.; Xie, T.; Zhang, D.; Leung, P.S. GPR120 Protects Lipotoxicity-Induced Pancreatic β-Cell Dysfunction through Regulation of PDX1 Expression and Inhibition of Islet Inflammation. Clin. Sci. 2019, 133, 101–116. [CrossRef]

74. Oh, D.Y.; Walenta, E.; Akiyama, T.E.; Lagakos, V.; Sun, Y.; Liu, J.; Luo, J.; Zhang, J.; et al. Seven Transmembrane G Protein-Coupled Receptor Repertoire of Gastric Ghrelin Cells. Mol. Metab. 2013, 2, 376–392. [CrossRef] [PubMed]

75. Stone, V.M.; Dhayal, S.; Brocklehurst, K.J.; Lenaghan, C.; Sörhede Winzell, M.; Hammar, M.; Xu, X.; Smith, D.M.; Morgan, N.G. GPR120 (FFAR4) Is Preferentially Expressed in Pancreatic Delta Cells and Regulates Somatostatin Secretion from Murine Islets of Langerhans. Diabetologia 2014, 57, 1182–1191. [CrossRef]

76. Engelstoft, M.S.; Park, W.M.; Sakata, I.; Kristensen, L.V.; Osbourne-Lawrence, S.; Piper, P.K.; Walker, A.K.; Pedersen, M.H.; Nehr, M.K.; et al. Seven Transmembrane G Protein-Coupled Receptor Repertoire of Gastric Somatostatin Cells. Endocrinology 2015, 156, 3909–3923. [CrossRef] [PubMed]
126. Zbieralski, K.; Wawrzycka, D. Alpha-Arrestins and Their Functions: From Yeast to Human Health. *Int. J. Mol. Sci.* 2022, 23, 4988. [CrossRef]

127. Patwari, P.; Lee, R.T. An Expanded Family of Arrestins Regulate Metabolism. *Trends Endocrinol. Metab.* 2012, 23, 216–222. [CrossRef]

128. Wedegaertner, H.; Pan, W.A.; Gonzalez, C.C.; Gonzalez, D.J.; Trejo, J. The α-Arrestin ARRDC3 Is an Emerging Multifunctional Adaptor Protein in Cancer. *Antioxid Redox Signal.* 2022, 36, 1066–1079. [CrossRef] [PubMed]

129. Peterson, Y.K.; Luttrell, L.M. The Diverse Roles of Arrestin Scaffolds in g Protein–Coupled Receptor Signaling. *Pharmacol. Rev.* 2017, 69, 256–297. [CrossRef] [PubMed]

130. McDonald, P.H.; Chow, C.-W.; Miller, W.E.; Laporte, S.A.; Field, M.E.; Lin, F.-T.; Davis, R.J.; Lefkowitz, R.J. β-Arrestin 2: A Receptor-Regulated MAPK Scaffold for the Activation of JNK3. *Science* 2000, 290, 1574–1577. [CrossRef] [PubMed]

131. Grundmann, M.; Merten, N.; Malfacini, D.; Inoue, A.; Preis, P.; Simon, K.; Rüttiger, N.; Ziegler, N.; Benkel, T.; Schmitt, N.K.; et al. Lack of Beta-Arrestin Signaling in the Absence of Active G Proteins. *Nat. Commun.* 2018, 9, 341. [CrossRef] [PubMed]

132. Yin, H.; Chu, A.; Li, W.; Wang, B.; Shelton, F.; Otero, F.; Nguyen, D.G.; Caldwell, J.S.; Chen, Y.A. Lipid G Protein-Coupled Receptor Ligand Identification Using Beta-Arrestin PathHunter Assay. *J. Biol. Chem.* 2009, 284, 12328–12338. [CrossRef] [PubMed]

133. Guzmán-Silva, A.; Martínez-Morales, J.C.; del Carmen Medina, L.; Romero-Avila, M.T.; Villegas-Comonfort, S.; Solís, K.H.; García-Sáinz, J.A. Mutation of Putative Phosphorylation Sites in the Free Fatty Acid Receptor 1: Effects on Signaling, Receptor Phosphorylation, and Internalization. *Mol. Cell. Endocrinol.* 2022, 545, 111573. [CrossRef]

134. Burns, R.N.; Singh, M.; Senatorov, I.S.; Moniri, N.H. Mechanisms of Homologous and Heterologous Phosphorylation of FFA Receptor 4 (GPR120): GRK6 and PKC Mediate Phosphorylation of Thr347, Ser350, and Ser357 in the C-Terminal Tail. *Biochem. Pharmacol.* 2014, 87, 650–659. [CrossRef]

135. Butcher, A.J.; Hudson, B.D.; Shimpukade, B.; Alvarez-curto, E.; Prihandoko, R.; Ulven, T.; Milligan, G.; Tobin, A.B. Concomitant Action of Structural Elements and Receptor Phosphorylation Determines Arrestin-3 Interaction with the Free Fatty Acid Receptor FFA4. *J. Biol. Chem.* 2014, 289, 18451–18465. [CrossRef]

136. Tobin, A.B. G-Protein-Coupled Receptor Phosphorylation: Where, When and by Whom. *Br. J. Pharmacol.* 2008, 153, 167–176. [CrossRef]

137. Senatorov, I.S.; Cheshmehkani, A.; Burns, R.N.; Singh, K.; Moniri, N.H. Carboxy-Terminal Phosphoregulation of the Long Splice Isoform of Free-Fatty Acid Receptor-4 Mediates β-arrestin Recruitment and Signaling to ERK1/2. *Mol. Pharmacol.* 2020, 97, 304–313. [CrossRef]

138. Bolognini, D.; Barki, N.; Butcher, A.J.; Hudson, B.D.; Sergeev, E.; Molloy, C.; Moss, C.E.; Bradley, S.J.; Le Gouill, C.; Bouvier, M.; et al. Chemogenetics Defines Receptor-Mediated Functions of Short Chain Free Fatty Acids. *Nat. Chem. Biol.* 2019, 15, 489–498. [CrossRef] [PubMed]

139. Milligan, G.; Barki, N.; Tobin, A.B. Chemogenetic Approaches to Explore the Functions of Free Fatty Acid Receptor 2. *Trends Pharmacol. Sci.* 2021, 42, 191–202. [CrossRef] [PubMed]

140. Barki, N.; Bolognini, D.; Börjesson, U.; Jenkins, L.; Riddell, J.; Hughes, D.I.; Ulven, T.; Hudson, B.D.; Ulven, E.R.; Dekker, N.; et al. Chemogenetics Defines a Short-Chain Fatty Acid Receptor Gut–Brain Axis. *Elife* 2022, 11, e73777. [CrossRef] [PubMed]