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SCFA Receptors in Pancreatic Beta Cells: Novel Diabetes Targets?

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Abstract

Nutrient sensing receptors are key metabolic mediators of responses to dietary and endogenously derived nutrients. These receptors are largely G-protein coupled receptors (GPCRs) and many are gaining significant interest as drug targets with a potential therapeutic role in metabolic diseases. A distinct subclass of nutrient sensing GPCRs, two short chain fatty acid (SCFA) receptors (FFA2 and FFA3) are uniquely responsive to gut microbiota derived nutrients (such as acetate, propionate and butyrate). Pharmacologic, molecular and genetic studies have investigated their role in organismal glucose metabolism and recently in pancreatic beta-cell biology. Here we summarize the present knowledge on the role of these receptors as metabolic sensors in beta cell function and physiology, revealing new therapeutic opportunities for type 2 diabetes.

Keywords

beta cell mass; FFA2; FFA3; gut microbiota; insulin secretion; short chain fatty acids

"Let food be thy medicine and medicine be thy food"- Hippocrates, 460 BC-370 BC.

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NUTRIENT SENSING RECEPTORS

Dietary nutrients beyond providing sustenance influence our health through immune and metabolic effects [1]. While their mode of action was previously thought to be through their intracellular metabolism [1], the discovery of membrane receptors that sense nutrients (**nutrient sensors,** see Glossary) has made it apparent that nutrients can act via these receptors to transmit cues to intracellular molecular pathways. These receptors generally belong to the G protein coupled receptor (GPCR) class [2], where the term 'nutrient sensing GPCRs' was originally suggested for the free fatty acid (FFA) receptors (see [2]). Along with FFA receptors (FFAR), nutrient sensing GPCRs also exist for amino acids [3] and carbohydrates [4].

The FFAR subclass binds to fatty acids or their derivatives, with each GPCR in this subclass exhibiting specificity for fatty acids (FAs) of a particular chain length. FFA1 (or GPR40), GPR84 and FFA4 (or GPR120) are activated by medium or long chain FAs (carbon length above 6); whereas, four other members of the FFAR family, FFA2 (previously GPR43), FFA3 (previously GPR41), GPR109a and OLFR78 bind short chain fatty acids (SCFAs, ranging from 1–6 carbons in length) [5, 6, 7]. While SCFAs are a source of energy and are primarily generated through gut **microbiota** fermentation, they also act systemically as signaling metabolites and gene transcription modulators (Box 1) [6]. The discovery of receptors specific to SCFAs has led to the attribution of the metabolic effects of the gut microbiota and SCFAs to these receptors. As the majority of published data in the SCFA receptor field has been on FFA2 and FFA3, we focus on these two GPCRs in this review (Table 1, see [7] for more details on GPR109a and OLFR78). Additionally, the potency of SCFAs for these other two receptors is much less than FFA2 and FFA3, and their role as SCFA receptors is just beginning to emerge [8, 9]. In the sections that follow, we highlight the basic pharmacology of FFA2 and FFA3, identify their biological roles with a focus on the pancreatic beta (β) cells, and explain how studies have begun to connect these GPCRs to the gut microbiota. The therapeutic potential of these receptors as novel metabolic targets for diabetes is also highlighted in the review.

BOX 1

The long and short on SCFAs

Acetate, propionate and butyrate, the most abundant SCFAs in the body, are produced by the gut microbiota where a large percentage of SCFAs are immediately metabolized in the colon. SCFAs not metabolized by colonocytes are absorbed into the hepatic and portal venous systems and emerge from liver into the systemic circulation [6]. Broadly, SCFAs contribute to many biological pathways, including having metabolic, immune, and intestinal effects. Metabolically, SCFAs are rich energy sources for colonocytes, liver, muscle, kidney as well as brain and heart [60]. Moreover, dietary SCFA supplementation in rodents diminishes obesity and increases insulin sensitivity and energy expenditure [61, 62]. Acetate and propionate can also modulate energy intake by reducing appetite via central mechanisms [59, 63]. Beyond metabolism, SCFAs have been reported to regulate colonic regulatory T cells (Treg) and promote differentiation of naive $CD4^+$ T cells to anti-inflammatory $FOXP3^+$ Tregs [64], that then migrate to target

tissues like brain and adipose suppressing inflammation and autoimmunity [65]. SCFAs also protect the integrity of the gut epithelium by competitively excluding the growth of pathogenic bacteria and preventing leakiness of the gut barrier [58]. Because of these findings, SCFAs have been considered as therapeutic modalities against intestinal disorders and against leaky gut derived metabolic endotoxemia associated with obesity, adipose inflammation and insulin resistance [66–68]. With these benefits, exploring the roles of SCFAs in coordinating various biological processes is an exciting area of research.

Pharmacology of Short Chain Fatty Acid Receptors, FFA2 and FFA3

Ligand selectivity—The SCFAs were identified as ligands for FFA2 and FFA3 over 10 years ago [10, 11]. Since then, a substantial effort has been placed into determining the potency of various SCFAs for both receptors. Prior to discussing these data, it is important to note what SCFA levels are in the blood, where the levels in humans and mice are similar and range from approximately 100–200 μM for acetate and 1–20 μM for propionate and butyrate [6, 12]. For FFA2, both acetate and propionate are the most potent agonists (Table 1) [10, 11, 13–15]. Longer SCFAs (4–7 carbons in length) and formate also show the ability to activate FFA2, albeit less potently than acetate or propionate [13–15]. With FFA3, slightly longer SCFAs have the greatest potency with valerate being the most potent, followed by butyrate [13], but this activity decreases as size increases to medium-chained acids. Another distinction is that SCFAs with branched alkyl groups such as isovalerate and isobutyrate tend to favor FFA3 over FFA2 [15]. An interesting observation is made when the alkyl chain is modified with unsaturated double or triple bonds [15]. When the double bond of the SCFAs is in the α,β-position, compounds have less potency for FFA3 while maintaining or increasing their effect on FFA2. The preference for FFA2 to bind acrylates and for FFA3 to be activated by branched alkanes suggests that the orthosteric binding pocket of FFA2 is smaller and possibly narrower than FFA3.

Role of FFA2 and FFA3 in Regulation of Metabolism

Owing to their expression in many metabolically relevant tissues, evaluation of the physiological roles of FFA2 and FFA3 is topical especially considering the emerging of role of the gut microbiota in metabolism. In the past one year, contributions of FFA2 and FFA3 to pancreatic β cell function including glucose stimulated insulin secretion (GSIS), β cell mass and to β cell responses to insulin resistance have been recognized [16–19], and this role of FFA2 and FFA3 in β cells is the primary focus of the following sections.

Islet expression of FFA2 and FFA3 and the influence of insulin resistance—Multiple initial observations suggested a role of both receptors in β cell function. First, Arena Pharmaceuticals reported in the patent literature that *Ffar2* and *Ffar3* levels were upregulated in the islets of *db/db* and *ob/ob*, and *db/db* mice, respectively [20, 21]. Subsequently, other groups observed increased expression of *Ffar2* in islets during the insulin resistant phase of pregnancy [22, 23]. Furthermore, a mouse gene database profiling in islets, adipose, skeletal muscle, and hypothalamus relative to obesity, diabetes susceptibility, and age [24] revealed increased islet specific expression of *Ffar2* in obese

mice, independent of type 2 diabetes (T2D) susceptibility [17]. Collectively, these data suggest that β cell expressed FFA2 and possibly FFA3 may contribute to the β cell response to insulin resistance.

Role of FFA2 and FFA3 in insulin secretion—A primary function of β cells is to secrete insulin, where its principal secretagogue is glucose. Besides glucose, others nutrients including SCFAs have been reported to modulate GSIS and prior to the discovery of nutrient-sensing receptors, it was thought to be through their intracellular metabolism [25]. A range of studies over the past 50 years have observed SCFAs contribute to GSIS (see [26–29]); however, there was no clear consensus whether the effects are stimulatory or inhibitory. During 2015, three groups independently reported on the role of FFA2 and FFA3 in β cells [12, 16–19] providing evidence that these GPCRs can, in part, be the mode that SCFAs influence GSIS.

Continuing with the trend prior to the discovery of these receptors, reported outcomes in 2015 of how these nutrients and their receptors mediate GSIS were divergent. Tang et al [16], through studies using both mouse (MIN6) and human pancreatic β cell lines (EndoC-βH1) and a combination of genetic FFA2 and FFA3 knockout mouse models and human islets, reported that FFA2 and FFA3 are negative modulators of GSIS (using acetate as the activator of both receptors). Moreover, each of these receptors was observed to signal via a pertussis toxin (PTX)-sensitive $G\alpha_{i/o}$ pathway (see Box 2). Priyadarshini and Layden [18] confirmed that FFA3 mediates a PTX-sensitive inhibition of GSIS using mouse islets, SCFAs and a FFA3 agonist to probe the function of FFA3. However, Priyadarshini et al [17] and McNelis et al [19] reported that FFA2 signaling largely augments GSIS (and does not diminish GSIS) and does this through the Gα subunit class, $G\alpha_{q/11}$. Next, we explore these opposing outcomes, which will help clarify these conflicting reports on how SCFAs and their GPCRs influence GSIS.

BOX 2

G protein binding profiles and signaling signatures

Following the deorphanization of these receptors [10, 11], investigation using yeast containing different yeast/mammalian Gα chimeras indicated that human FFA2 (hFFA2) activates Gpa1p/Gα chimeras containing the C termini of mammalian $G\alpha_{12}$, $G\alpha_{13}$, $G\alpha_{14}$, $G\alpha_{i1}$, and $G\alpha_{i3}$, suggesting that FFA2 activates the $G\alpha_{i/o}$, $G\alpha_{q/11}$, and $G\alpha_{12/13}$ families of G proteins. Additionally, acetate provoked a dose-dependent increase in (^{35}S)GTPγS binding in membranes prepared from HEK293T cells transfected with $G\alpha_o$ and hFFA2 [10, 11]. Le Poul et al [32] found that acetate or propionate promote FFA2 signaling to $G\alpha_{q/11}$ and $G\alpha_{i/o}$, through intracellular calcium and cAMP, respectively, in CHO-K1 and COS-7 cells expressing hFFA2. A (^{35}S)GTPγS binding assay confirmed acetate and propionate coupling of FFA2 with $G\alpha_{i/o}$ in a cell-free assay, and pertussis toxin (PTX) inhibited the response [32]. In cells cotransfected with FFA2 and $G\alpha_{qi5}$, there was an increase in the basal level of inositol phosphates over cells expressing only one of the plasmids, suggesting that FFA2 may have constitutive activity [32].

Despite amino acid sequence homology of FFA3 to FFA2, FFA3 couples exclusively through the PTX-sensitive $G\alpha_{i/o}$ family. Like FFA2, FFA3 was found to be constitutively active in yeast when co-expressed with Gpa1p/$G\alpha_{i1}$ and Gpa1p/$G\alpha_{i3}$ chimeras [10, 11]. FFA3 also responded to SCFAs when co-expressed in yeast with the Gpa1p/$G\alpha_{o}$ chimera, an effect not seen with FFA2 [10, 11]. Using transient co-transfection of hFFA3 and $G\alpha_{i3}$ into HEK293T cells and transfection of a hFFA3-$G\alpha_{i3}$ fusion protein, it was shown that propionate increased levels of $G\alpha_{i3}$-bound (^{35}S)GTPγS in a concentration-dependent manner [13]. Thus, FFA2 can couple to both $G\alpha_{q/11}$ and $G\alpha_{i/o}$ protein classes whereas FFA3 can couple to $G\alpha_{i/o}$ protein class. With different G protein binding profiles, these receptors can uniquely affect diverse cellular processes with either antagonistic or synergistic outcomes.

Co-existence of these receptors in pancreatic β cells suggests that their activation could differently affect insulin secretion (Figure 1) and cell mass (Figure 2). For example, FFA2 signaling via two divergent G protein classes ($G\alpha_{q/11}$ and $G\alpha_{i/o}$) is anticipated to have dissonant effects on GSIS, FFA3 coupling solely to $G\alpha_{i/o}$ class would inhibit GSIS [69].

First, Priyadarshini et al [17] showed that acetate (at 1 mM) potentiated GSIS from wild type (WT) islets as compared to islets from FFA2 null (*Ffar2*$^{-/-}$) mice in static insulin secretion studies. As these data conflicted with Tang et al [16], synthetic FFA2 agonists were used to probe the role of FFA2 in GSIS with mouse islets and it was observed that each had varying effects on GSIS; two agonists [15] potentiated GSIS in a FFA2-specific manner, while two other ligands CMTB (4-chloro-α-(1-methylethyl)-N-2-thiazolylbenzeneacetamide) [30] and PA ((S)-2-(4-chlorophenyl)-3,3-dimethyl-N-(5-phenylthiazol-2-yl)butanamide) [31]), both at 100 μM, inhibited GSIS from WT mouse islets. However, the inhibitory effects of CMTB and PA on GSIS were not exclusively through FFA2, indicating off-target effects. Further data through using pharmacological inhibitors of the respective G protein pathways showed that these FFA2 agonists mediate GSIS through coupling with $G\alpha_{q/11}$ and likely $G\alpha_{i/o}$ in β cells (Figure 1 & Box 2) [32]. The other study, McNelis et al [19], observed that FFA2 mediates GSIS through a $G\alpha_{q/11}$ dependent manner, where these results were obtained through studies with human islets, mouse islets, and MIN6 cells. However, they did not observe acetate (using 1 mM acetate) influenced GSIS but did find that PA (at 1 μM) stimulated insulin release in a $G\alpha_{q/11}$ dependent manner. Additionally, coupling of FFA2 to $G\alpha_{q/11}$ was further confirmed by PA-mediated accumulation of inositol-1,4,5-triphosphate (IP_3), a second messenger downstream of $G\alpha_{q/11}$ (Figure 1). Emerging from these two studies [17,19] is the important finding that FFA2 predominantly couples to $G\alpha_{q/11}$, but both groups did observe evidence that FFA2 also couples $G\alpha_{i/o}$.

While these two studies concluded that FFA2 mostly couples with $G\alpha_{q/11}$, differences existed between these studies which warrant exploration, in particular in the observed function of FFA2 in human islets. With human islets, Priyadarshini et al [17] showed data that acetate (at 1 mM) and SCA14, SCA15 and PA (each at 100 μM) did not affect GSIS, but the agonist, CMTB (at 100 μM), inhibited GSIS and that this inhibition was not PTX-sensitive. McNelis et al [19] showed PA (at 1 μM) augments GSIS with human islets in a

PTX-insensitive manner. Additional data from Priyadarshini et al [17] confirmed that the FFA2 agonists used in their study can simultaneously activate both $G\alpha_{q/11}$ and $G\alpha_{i/o}$ pathways in hFFA2 expressing CHO-K1 cells, suggesting the possibility that in human β cells, these agonists activate both pathways equally resulting in a net neutral effect on GSIS. Reasons for these different outcomes could be related to receptor pharmacology of FFA2 between humans and mice (see Box 3) or the lack of well characterized FFA2 agonists (see [33]). Additional investigations are needed to reconcile the differences in the human data between Priyadarshini et al [17] and McNelis et al [19] and to confirm the G-protein coupling preference in human islets. Another variable is that each of these human studies had only a small amount of donors, and differences very well may exist between islets from different donors in their response to different agonists (also, receptor expression may be influenced by unforeseen factors influencing receptor function). Further understanding of FFA2 signaling in human islets will ultimately be crucial to truly understand the therapeutic value of FFA2 in T2D.

BOX 3

Structural features define species specificity in receptor function

It has been observed that differences exist in the selectivity and potency of SCFAs binding to FFA2 and FFA3 orthologs between species. For example, the activities of the human and mouse FFA2 (hFFA2 and mFFA2) orthologs show clear differences, with acetate being nearly 100x more potent against hFFA2 than hFFA3, but nearly equipotent against the two mouse orthologs [14]. Additionally, while propionate has similar potency for hFFA2 and hFFA3, it is approximately 10x more potent for mFFA3 than mFFA2.

While the crystal structures of FFA2 and FFA3 have not been solved, there is substantial mutagenesis data that provides information on the binding mode of SCFAs to these receptors (Figure I). One identified interaction between receptor and ligand is with the carboxylate group of SCFAs [13, 14, 70]. Specifically, interactions between the carboxylate groups (which are negatively charged at physiological pH) with the positively charged arginine residues in the orthosteric site of FFA2 have been identified. In particular, Arg180 and Arg255 are essential for acetate or propionate agonist activity towards FFA2. It was also found that His242 is required for FFA2 activation by SCFAs, presumably due to a favorable electrostatic-driven complex between His242 and these two arginine side chains in the orthosteric pocket. Interestingly, the recently reported FFA1 crystal structure [71] shows strong interactions between the carboxylate of the bound ligand (TAK-875) and Arg183 and Arg258. These two arginine residues are equivalent to Arg255/180 in FFA2, confirming this orthosteric carboxylate binding interaction. These structural data have been useful in identifying why SCFAs have unique interactions with hFFA2 and mFFA2. Notably, a third residue in mFFA2 was identified which has a significant effect on the potency of SCFAs against FFA2. In mFFA2, a Glu159 forms an “ionic lock” with Arg255/180 in a manner similar to that of FFA1 [14]. However, in hFFA2, this glutamate is replaced by glycine, abolishing the ionic interaction. Mutagenesis studies confirmed that this residue plays a critical role in determining the species selectivity towards SCFAs between mouse and human FFA2.

These data are highly relevant because they may confound drug discovery efforts due to the reliance on non-human models [72].

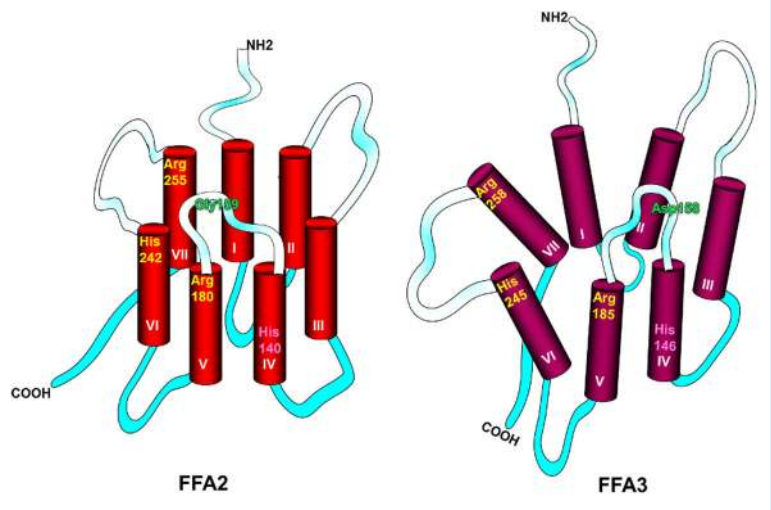

Figure I.

Key features of ligand binding sites of hFFA2 and hFFA3.

The binding pocket of FFA2 is smaller than FFA3, which conforms to the size selectivity of each receptor. Residues that play important role in SCFA recognition and binding are Arg 180 in FFA2 (Arg 185 in FFA3) at the top of transmembrane domain V (shown in yellow), His 242 in FFA2 (His 245 in FFA3) at the top of transmembrane domain VI and Arg 255 in FFA2 (Arg 258 in FFA3) at the top of transmembrane domain VII. These positively charged residues interact with the negatively charged carboxylate group of SCFAs facilitating their function. His 140 (His 146 in FFA3) (shown in pink) predicted to be lower in the binding pocket possibly plays a role in fatty acid chain length selectivity. Presence of an acidic non-conserved residue in extracellular loop 2 (connecting domains IV and V) is responsible for the variation in constitutive, ligand independent activity between human and rodent receptors. In orthologs lacking constitutive activity (mFFA2 and hFFA3), an extracellular ionic lock is formed between the arginine residues of the orthosteric binding site and an acidic residue in extracellular loop 2 (Glu159 in mFFA2 and Asp158 in hFFA3) whereas orthologs with high constitutive activity (hFFA2 and mFFA3 have Gly and Asn at corresponding positions, respectively) do not possess this interaction. For further explanation, see [13, 14, 15]. Green= residues that vary between the orthologs and play role in constitutive activity of the receptor. TM, transmembrane. Shaded segments= extracellular loops connecting the TM domains; solid aqua segments= intracellular loops.

As noted earlier in this review, insulin resistant states including that induced by high fat diet (HFD) influences receptor expression in islets in particular for FFA2 [17, 19]. An important question these data raises is whether this expression change alters receptor function, and insight into this possibility has been suggested. From Priyadarshini et al [17], islets from HFD fed *Ffar2*$^{-/-}$ mice showed a similar GSIS pattern to islets from normal chow fed mice

in the presence of the FFA2 agonists, with the exception of one of the FFA2 agonists, PA, which increased GSIS in islets from WT mice fed a HFD, as opposed to its inhibitory effect on GSIS in islets from WT normal chow fed mouse islets. These data provide evidence of a possible change in G-protein coupling with FFA2 from HFD. Next, measuring IP_3 and cAMP levels subsequent to PA stimulation, McNelis et al [19] observed that cAMP inhibition was similar in islets from HFD fed mice either on short term (8 week) or long term (14 week) regimen, but IP_3 accumulation increased as function of time on HFD, where the authors postulated that for FFA2, the ratio of stimulatory to inhibitory G-protein signaling may depend upon the duration of HFD consumption. Considering these data [17, 19], we suggest a shift in the G-protein coupling preference of FFA2 (or changing in coupling) may be occurring, in the context of HFD. Moreover, our lack of understanding of the G-protein coupling preferences of FFA2 may explain differences between each of these studies. Taken together, it is apparent from these studies that both FFA2 and FFA3 regulate GSIS, where FFA2 does this through $G\alpha_{q/11}$ and $G\alpha_{i/o}$, and FFA3 through $G\alpha_{i/o}$; however, a greater understanding is needed into what controls FFA2 coupling preferences.

Role of FFA2 and FFA3 in pancreatic β cell mass regulation—As with GSIS, $G\alpha_{q/11}$ coupled GPCRs have been observed to augment β cell mass expansion in response to insulin resistance, whereas $G\alpha_{i/o}$ coupled GPCRs have been reported to restrict the β cell mass expansion [34, 35]. Not surprisingly, McNelis et al [19] observed reduced β cell mass in *Ffar2*$^{-/-}$ mice on a HFD compared to WT mice, lower β cell proliferation and reduced expression of insulin and the β cell transcription factors (*Mafa*, *Pdx1*, *NeuroD*) [19]. Consistent with these findings, PA or acetate stimulated the proliferation of MIN6 cells, insulin levels, and *Pdx1* and *NeuroD* gene transcription, and was mediated via $G\alpha_{q/11}$ (Figure 2, **Key Figure**). Likewise, one of the other groups reported that *Ffar2*$^{-/-}$ mice exhibited reduced β cell mass and β cell proliferation in response to pregnancy [12]; however, their mouse model had reduced β cell mass prior to HFD and also pregnancy. As opposed to these studies, Tang et al [16] observed no morphological difference in their FFA2 or FFA3 knockout islets, but directly measuring β cell mass was not done. While these data suggest a role for FFA2 in the regulation of β cell mass, the role, if any, of FFA3 remains unknown, as it has not been reported.

Role of FFA2 and FFA3 in insulin resistance—Considering the diverse outcomes published above, it is not surprising that diverse *in vivo* phenotypes have also been reported on the role of FFA2 and FFA3 in β cell function during insulin resistant states. For FFA2, Fuller et al, Priyadarshini et al and McNelis et al [12, 17, 19] in different models of insulin resistance, each observed an *in vivo* insulin secretion deficit. During the insulin resistant phase of pregnancy, at gestation day 15 (G15) [36], *Ffar2*$^{-/-}$ mice exhibited an *in vivo* insulin secretory defect, specifically, pregnant *Ffar2*$^{-/-}$ mice exhibited impaired glucose tolerance due to low glucose stimulated insulin secretion [12]. However, this group did not observe with male *Ffar2*$^{-/-}$ mice on a HFD impaired glucose tolerance; but did observe an *in vivo* deficit during **hyperglycemic clamp** studies while mice were on normal chow [17]. McNelis et al [19] showed an impaired glucose tolerance with diminished *in vivo* insulin secretion on HFD for *Ffar2*$^{-/-}$ mice. However, Tang et al [16], observed the opposite, *Ffar2*$^{-/-}$ and *Ffar3*$^{-/-}$ mice or *Ffar2*$^{-/-}$;*Ffar3*$^{-/-}$ double knockout mice on HFD exhibited

increased glucose tolerance and plasma insulin levels post glucose challenge, with the effects being more pronounced in the double knockouts. Why these opposing outcomes are observed is not clear, but we suspect this is due to our lack of understanding of the G-protein coupling with FFA2 and/or the confounding variable of FFA2 and FFA3 expression in tissues other than islets (Figure 2).

Non-pancreatic beta cell roles in metabolism—FFA2 and FFA3 can also influence metabolism through their expression in other tissues and cell types, for example immune cells (see [37]), adipose tissue, neural tissue and gut L cells. A role of these receptors in gut L cells emerged from studies with *Ffar2*$^{-/-}$ and *Ffar3*$^{-/-}$ mice, either on chow [12, 38] or on HFD [19] with these mice having decreased secretion of GLP-1 in response to an oral glucose challenge. McNelis et al [19] also reported lower circulating gastric inhibitory polypeptide (GIP) and PYY (peptide YY) in the *Ffar2*$^{-/-}$ mice, hormones involved in regulating β cell function and suppressing appetite, respectively. An incretin role for these receptors raises the concern that some of the β cell function can partly be attributed to this function. Thus it is necessary to distinguish the roles of FFA2 and possibly FFA3 in β cells versus L cells. It is important to mention, however, the roles of these receptors in incretin release *in vivo* has not been observed by all groups [16].

A role of FFA2 and FFA3 in adiposity has been reported, but is also debated. Early reports observed a reduction of body fat mass in *Ffar2*$^{-/-}$ mice on HFD, from altered energy expenditure [39]. These findings are directly contradicted by others [40], where it was observed that FFA2 inhibits insulin signaling and hence fat accumulation in adipose tissue. In this study [40], FFA2 ablation led to spontaneous obesity on normal chow, whereas overexpressing *Ffar2* in adipose tissue led to a lean phenotype on a HFD, effects that were attributed to the gut microbiota. It is noteworthy that FFA2 has also been reported to increase leptin and decrease ghrelin secretion, two hormones that influence energy balance [41, 42]. FFA3 signaling has also been implicated in modulating body fat mass, although these effects are also far from clear with reports of *Ffar3*$^{-/-}$ mice having no change [38], loss [43] or gain of body fat mass [44]. In summary, contradictory results have also been obtained with both FFA2 and FFA3 knockout models on their role in the development of adiposity.

These receptors are also reported to influence the immune system, and for FFA3, in the nervous system. For example, *Ffar2*$^{-/-}$ mice exhibit exacerbated colitis, arthritis and asthma [45], while the stimulation of FFA2 affects immune cell recruitment, production of chemokines and cytokines [45–49]. These results indicate a role for FFA2 in inflammation, where the action of this receptor can seemingly be both pro- and anti-inflammatory, depending on the specific cell type [37]. Likewise, FFA3 has been reported to influence inflammatory responses in intestinal epithelial cells and lungs [50, 51]. And finally, FFA3 is expressed in sympathetic ganglion and the enteric nervous system [52, 53]. In the fed state, FFA3 agonism has been reported to increase energy expenditure via sympathetic outflow [52, 54]; while under fasting conditions, FFA3 signaling attenuates energy expenditure and in sympathetic outflow. FFA3 activation in peripheral nerves by propionate was also reported to have metabolic benefits such as improved glucose control, decreased adiposity, and decreased hepatic glucose production through increased intestinal gluconeogenesis [55]. Collectively, signaling of FFA2 and FFA3 in adipose, gut, neural, pancreatic β cells and

inflammatory cells can affect overall metabolic homeostasis (Figure 2). As apparent, the conflicting data might arise from the multitude of effects at these different tissues, but, as discussed next, may also be affected by gut microbial activity through undefined mechanisms.

Considerations in understanding the gut microbiota to receptor relationship

Specific compositional and functional gut microbial signatures are associated with particular host metabolic phenotypes [56, 57]. One mechanism by which gut microbiota influences host metabolism is through production of metabolites, like SCFAs [58] which as highlighted above, can influence host physiology by their ligation with FFA2 and FFA3 (Box 1 & Figure 2). Unraveling this interface between gut microbiota and metabolic phenotypes through these receptors is emerging from rodent studies using receptor knockout models, models of **gnotobiotic** mice and from dietary intervention models [12, 19, 40, 55]. One example is that *Ffar3*$^{-/-}$ gnotobiotic or conventionally raised mice were leaner than WT mice due to reduced PYY levels, increased gut transit and decreased energy harvest from SCFAs [43], and this effect was influenced by the gut microbiota. Similar effects have been published by Kimura et al [40] where *Ffar2*$^{-/-}$ mice and HFD fed mice over expressing *Ffar2* in adipose tissue had altered weight phenotypes that reverted to normal under germ free conditions or with antibiotic treatment. Metabolic phenotype and diet associated gut microbial shifts in *Ffar2* mouse models have also been depicted in other studies [12, 19]. On a HFD, WT and *Ffar2*$^{-/-}$ mice showed distinct fecal bacterial phyla profiles with an observed increase ratio of Firmicutes/Bacteroidetes, a possible signature for human obesity [19]. Functional impacts of these changes in the gut microbiota in the WT and *Ffar2*$^{-/-}$ mice on HFD led to higher serum acetate levels [19].

The exact implication of this relationship between the gut microbiota and SCFAs through these receptors at the β cells remains unclear. In a pregnancy mouse model, where pregnancy is a state of insulin resistance, Fuller et al [12] provides an initial exploration of this relationship. While no significant overall gut microbial shifts were observed [12], changes in relative abundances of Actinobacteria and Bacteroidetes in the postpartum period occurred in WT mice. Also, functional changes were evident in plasma SCFA levels with acetate trending higher and propionate lower during pregnancy, which could be relevant to FFA2 activity, as acetate is a more potent ligand than propionate. Moreover, *Ffar2*$^{-/-}$ mice had gestational impaired glucose tolerance, possibly due to the absence of acetate-FFA2 signaling during pregnancy, which led to impaired *in vivo* insulin secretion and β cell proliferation [12]. As with other tissues that express FFA2 and FFA3, effects of gut microbial generated SCFAs may be influencing FFA2 and FFA3 function in pancreatic β cell function and growth [12, 16, 17, 19], thus representing a novel ‘gut/β cell axis’.

Concluding Remarks and Future Perspectives

FFA2 and FFA3 impact metabolism through their roles in β cells and other tissues. Complicating the understanding of the function of these receptors is the fact that FFA2 (and possibly FFA3) couple to multiple G proteins *in vivo* and exhibit species specific responses to ligands [19] (Box 2 and Box 3). Likewise, SCFAs exhibit functional redundancy for these receptors (Box 3) and also, SCFAs likely exert effects independent of these receptors [57,

59] (Box 1). Another challenging variable with the mouse models being used to decipher the function of these receptors is that FFA2 and FFA3 genes are located in close genetic proximity, thus, genetic knockout may modulate the expression of the other receptor [18, 42]. This calls for additional, more specific, tissue knockout models. To support these models, the development of selective specific synthetic agonists and antagonists that are characterized for species- and signaling-preferences are needed for detailed dissection of the physiological roles of these receptors. Furthermore, differences in the gut microbiota composition (between animal facilities) may lead to discordant phenotypes of these mouse models possibly through altering SCFA levels and consequently the function of these receptors; thus to eliminate this variable it may be necessary to control for the gut microbiota through gnotobiotic conditions when investigating these receptors.

Studies defining the pharmacology and metabolic function of FFA2 and FFA3 are yielding our initial understanding of the role of these receptors in the β cell and beyond. It is now demonstrated that FFA2 and FFA3 modulate GSIS, and FFA2 contributes to the regulation of β cell mass. Consistent evidence is still lacking and key questions remain (see Outstanding Questions Box). Moving forward, manipulation of FFA2 and/or FFA3 function in the β cells as targets for diabetes therapy still requires validation in animal models, with close translation to human tissue.

Outstanding Questions

What are the main functions of FFA2 and/or FFA3 outside the β-cells, and how do these functions influence aspects of β-cell biology?

What factors regulate expression of FFA2 and FFA3 in β cells and outside of β cells?

Does increased mRNA expression of FFA2 and FFA3 lead to altered function?

What physiological and pathological conditions mediate Gα coupling preference for FFA2?

While FFA2 activation may be an initial adaptive mechanism under states of insulin resistance to compensate for increase in insulin requirements, what are the effects of its chronic activation?

Pharmacological studies suggest differences exist in the constitutive activity of each receptor. How does this constitutive activity influence their function in β-cells?

How do FFA2 and FFA3 mouse studies translate to humans?

Are biased FFA2-$G\alpha_{q/11}$ agonists and/or FFA3 antagonists a reasonable approach to treat type 2 diabetes?

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Glossary

Gnotobiotic	is an organism with a defined microbial community such as germ free or colonized with a particular microbial species.
Hyperglycemic clamp	involves clamping the plasma glucose concentration at a pre-specified elevated level through a specific glucose infusion rate, which is dependent on β cell's ability to secrete insulin and the body's ability to metabolize glucose.
Insulin sensitivity	is the measure of responsiveness of organs to metabolic actions of insulin. Significantly reduced insulin sensitivity is used as prognostic parameter for type 2 diabetes and metabolic syndrome.
Microbiota	represents the microbial community of a specific region.
Nutrient sensors	are functionally and structurally diverse molecular machines either transmembrane (GPCRs) or cytosolic (AMPK, mTOR) that are specialized in detecting specific nutrients. Detection of the nutrient is followed by induction of a response, which may alter cellular physiology.

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Trends Box

FFA2 and FFA3, uniquely respond to ligands that are derived from the gut microbiota and thus, represent a biologic mechanism linking microbial composition (and its changes) to glycemic control and multi-tissue homeostasis.

FFA2 and FFA3 are novel effectors of glucose homeostasis in part due to their direct effects on insulin secretion and beta cell proliferation.

Associated challenges in establishment of the biology of these receptors are from the scarcity of selective agonists, insufficient mouse models and challenges in correlating changes in endogenous ligands of these receptors to specific gut microbial/dietary/metabolic profiles.

The still evolving novel gut-pancreas axis represents a potential therapeutic target for treatment of type 2 diabetes and related metabolic disorders.

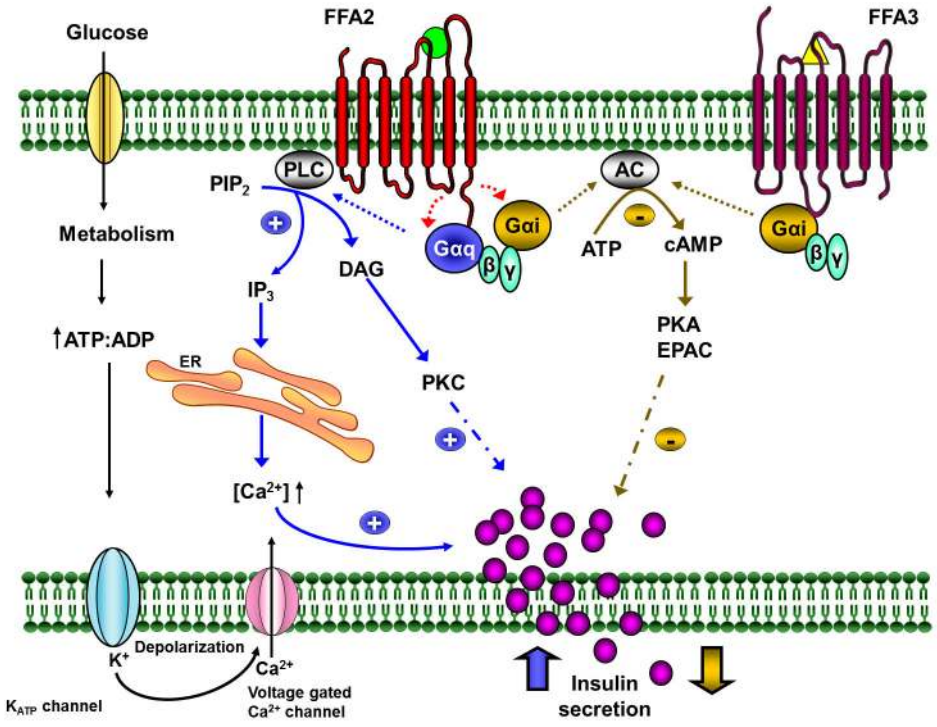

Figure 1.

Modulation of insulin secretion by FFA2 and FFA3.

Glucose once internalized in β cells is metabolized which results in elevation of ATP:ADP ratio, closure of K_{ATP} channels, cell membrane depolarization, opening of voltage dependent calcium channels (Ca^{2+} channels) causing calcium influx triggering insulin vesicle exocytosis. SCFAs (acetate and propionate) can bind to FFA2 and FFA3 either amplifying (in blue) or diminishing (in golden) glucose stimulated insulin secretion. Upon ligand activation of FFA2, $G\alpha_{q/11}$ subunits activate PLC which hydrolyses PIP_2 to DAG and IP_3 which in turn activates PKC and releases calcium from ER stores, respectively, amplifying the insulin release. FFA2, like FFA3, can also couple with $G\alpha_{i/o}$ subunits and inhibit AC, which decreases the concentration of cAMP, inhibiting PKA and EPAC mediated insulin release.

Abbreviations: AC, adenylate cyclase; cAMP, cyclic AMP; DAG, diacylglycerol; EPAC, exchange protein directly activated by cAMP; ER, endoplasmic reticulum; FFA2, free fatty acid receptor 2; FFA3, free fatty acid receptor 3; IP_3, inositol triphosphate; K_{ATP} channels, ATP sensitive potassium channels; PLC, phospholipase C; PIP_2, phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C; PKA, protein kinase A; SCFAs, short chain fatty acids. Green circle for acetate and yellow triangle for propionate.

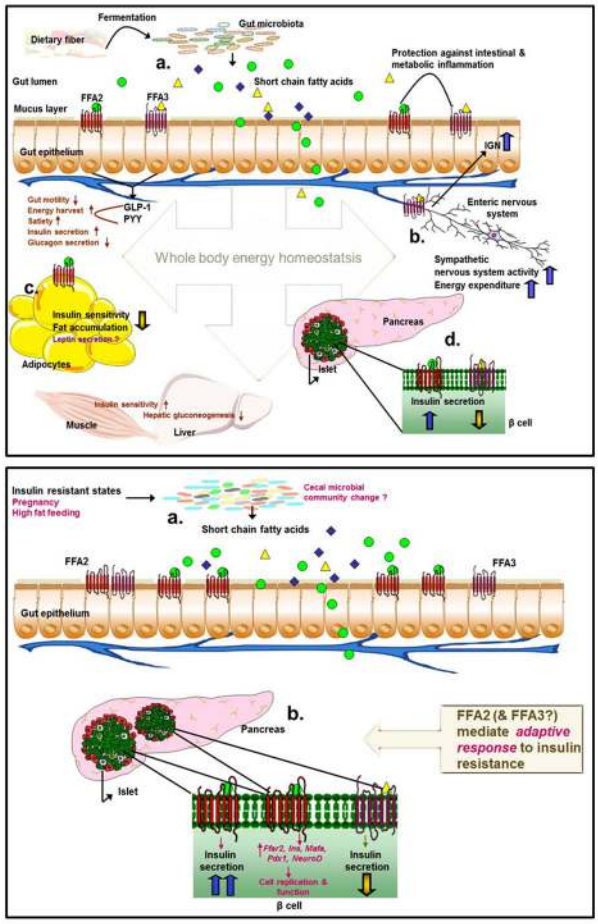

Figure 2.

Homeostatic role of FFA2 and FFA3 in energy metabolism, a global view (upper panel) and β cell-specific view (lower panel).

Gut microbial fermentation products, SCFAs, activate FFA2 and -3 on enteroendocrine L cells stimulating the release of GLP-1 and PYY, which affect energy absorption and metabolism via effects on gut function and outside the gut (a). Activation of FFA3 on the enteric neurons and sympathetic ganglia enhances intestinal gluconeogenesis and sympathetic outflow, reducing hepatic glucose production and increasing energy expenditure (b). Stimulation of FFA2 decreases insulin signaling in adipocytes with a consequent increase in energy expenditure in other tissues (c). In β cells, activation of FFA2 or FFA3 causes opposing affects on insulin secretion (d). Lower panel: Under states of insulin resistance (pregnancy and high fat feeding), gut microbiota composition changes may lead to altered cecal and plasma levels of SCFAs (a). In β cells, increased expression and activation of FFA2 causes compensatory increase in insulin secretion and transcription of factors responsible for increased β cell proliferation and mass, contributing to adaptive responses of the β cell to insulin resistance. The role of FFA3 in insulin resistance is less clear (b).

Abbreviations: IGN, intestinal gluconeogenesis; Ins, insulin; Mafa, v-maf musculoaponeurotic fibrosarcoma oncogene family, protein A; NeuroD, neuronal differentiation 1; Pdx1, pancreatic and duodenal homeobox 1; PYY, peptide YY; SCFAs, short chain fatty acids. Green circles= acetate; yellow triangles= propionate; blue diamonds= butyrate. Dark brown: effects secondary to receptor activation; purple: unclear roles of receptor signaling; magenta: insulin resistance mediated changes.

Table 1

Summary of the short chain fatty acid receptors (FFA2 and -3), highlighting their tissue expression, endogenous and synthetic ligands, G-protein signaling/effector, and biological functions

Receptor	Tissue expression	Endogenous ligands	Synthetic agonists	G protein & effector	Biological functions[a]	Ref
FFA2	Pancreatic islets, white adipocytes, enteroendocrine cells, immune cells	SCFAs (C2-C6)[a] acetate (pEC_{50} 4.5/3.6)[b] propionate (pEC_{50} 4.2/3.7)[b]	CMTB (IC_{50} 0.7/1.0)[b] PA (IC_{50} 0.7/NA)[b] SCA14 (pEC_{50} 3.2/NA)[b] SCA15 (pEC_{50} 2.7/NA)[b]	$G\alpha_{i/o}$/cAMP , $G\alpha_{q/11}/Ca^{2+}$	Insulin secretion ↑	[11, 17, 19, 37–42, 45–49, 53]
					GLP-1 secretion ↑	
					GIP secretion ↑	
					ghrelin secretion ↓	
					leptin secretion ?	
					PYY secretion ↑	
					β cell growth ↑	
					insulin signaling in adipose ↓	
					regulation of inflammatory responses	
					gut homeostasis ↑	
FFA3	Pancreatic islets, enteroendocrine cells, sympathetic ganglia, enteric neurons, immune cells, white adipocytes	SCFAs (C3-C6)[a] valerate (pEC_{50} 4.6/NA)[b] butyrate (pEC_{50} 4.0/3.7)[b] propionate (pEC_{50} 4.1/4.9)[b]	MCPC (pEC_{50} 3.9/4.3)[b]	$G\alpha_{i/o}$/cAMP	Insulin secretion ↓	[16, 18, 38, 43, 44, 46,50–55]
					GLP-1 secretion ↑	
					PYY secretion ↑	
					intestinal gluconeogenesis ↑	
					sympathetic outflow regulation	
					inflammation ↓	

[a] carbon chain length of SCFAs and particular SCFAs with a preference; ↑, promotes; ↓, decreases, ?, controversial

[b] pEC_{50} or IC_{50} values for human receptor/mouse receptor as available from various functional assays [14, 15, 30, 31]

NA, not available

CMTB, 4-chloro-α-(1-methylethyl)-N-2-thiazolylbenzeneacetamide)

PA, ((S)-2-(4-chlorophenyl)-3,3-dimethyl-N-(5-phenylthiazol-2-yl)butanamide)

SCA, small carboxylic acid

MCPC, 1-methylcyclopropanecarboxylic acid