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Review

# Free Fatty Acid Receptor 1: A New Drug Target for Type 2 Diabetes?

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ABSTRACT

Free fatty acid receptor 1/G-protein-coupled receptor 40 (FFA1/GPR40) is activated by medium- to long-chain fatty acids (FA) and preferentially expressed in pancreatic β-cells. GPR40 mediates the acute potentiating effect of FA on glucose-stimulated insulin secretion, but not their chronic deleterious effects. As such, GPR40 is being considered as a new therapeutic target to enhance insulin secretion in type 2 diabetes mellitus. A number of preclinical studies and recent phase 2 clinical trials support the beneficial effects of a GPR40 agonist in type 2 diabetes. Recent studies from our laboratory identified protein kinase D as a downstream target of GPR40, which regulates cortical actin remodelling and amplifies the second phase of insulin secretion in response to fatty acids. We have also observed that glucose regulates the expression of the gene encoding GPR40 via a transcriptional mechanism that involves O-GlcNAcylation of the transcription factor pancreas-duodenum homeobox-1 and requires activity of phosphatidylinositol-3-kinase. These recent studies provide important mechanistic information as GPR40 agonists are being developed as new type 2 diabetes drugs; however, many questions remain to be answered regarding the biology of this receptor and its potential role in tissues other than the β-cell.



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RÉSUMÉ

Le récepteur couplé aux protéines G FFA1/GPR40 est activé par les acides gras (AG) à chaînes moyenne et longue, et est préférentiellement exprimé dans les cellules bêta-pancréatiques. GPR40 est responsable de l'effet potentialisateur des AG à court terme sur la sécrétion d'insuline en réponse au glucose, mais non de leurs effets délétères à long terme. À ce titre, GPR40 est considéré comme une nouvelle cible thérapeutique pour améliorer la sécrétion d'insuline dans le diabète de type 2. Plusieurs études précliniques et de récents essais cliniques de phase 2 corroborent les effets bénéfiques d'un agoniste de GPR40 sur l'homéostasie glycémique. De récentes études dans notre laboratoire ont identifié la protéine kinase D comme une cible intracellulaire activée en aval de GPR40, qui régule le remodelage de l'actine corticale et amplifie la seconde phase de sécrétion d'insuline en réponse aux AG. Nous avons également observé que le glucose régule l'expression du gène codant pour GPR40 par un mécanisme transcriptionnel qui implique la glycosylation du facteur de transcription PDX-1 et requiert l'activité de la phosphatidylinositol-3-kinase. Ces récentes études apportent des informations importantes quant aux mécanismes d'action de GPR40, tandis que des agonistes de ce récepteur sont en cours de développement pour le traitement du diabète de type 2. Cependant, plusieurs questions restent à explorer concernant la biologie de GPR40 et son rôle potentiel dans les tissus autres que la cellule bêta.



## Introduction

Type 2 diabetes mellitus is a global public health crisis and is expected to affect over 550 million people worldwide in 2030. Type 2 diabetes occurs when pancreatic β-cells are unable to compensate for insulin resistance induced by environmental factors such as obesity. Thus, insulin secretory defects are an integral part of the

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pathogenesis of type 2 diabetes, and as such one of the therapeutic approaches to treat type 2 diabetes is aimed at enhancing insulin secretion from pancreatic β-cells. To avoid iatrogenic hypoglycemia, such drugs should only be effective when circulating glucose levels are elevated.

Long-chain fatty acids (FA) do not initiate insulin secretion at low or normal glucose levels, but strongly potentiate glucose-induced insulin secretion (GSIS). Identification of the G-protein coupled receptor (GPCR) free fatty acid receptor 1/G-protein-coupled receptor 40 (FFA1/GPR40) as a medium- to long-chain FA receptor predominantly expressed in β-cells (1–3) and that mediates in large part the potentiation of GSIS by FA (2,4,5) sparked tremendous interest in the potential of this receptor as a novel drug target for type 2 diabetes treatment. A number of GPR40 agonists are under development as type 2 diabetes drugs, including one that showed promising results in recent phase 2 clinical trials (6,7).

### *From GPR40 physiological studies to the development of new type 2 diabetes drugs*

Several groups have investigated the role of GPR40 in β-cell function, and the results from these studies concur to show that GPR40 plays a key role in mediating the potentiation of GSIS by FA. In different lines of mice with whole-body deletion of the gene encoding GPR40 (GPR40$^{-/-}$), a strong reduction of the potentiation of GSIS in response to acute stimulation by Intralipid in vivo or to medium- to long-chain saturated and unsaturated FA in vitro was observed, without changes in GSIS itself or other apparent defects under unchallenged conditions (5,8–10). Similar effects were seen in insulin-secreting cell lines transfected with small interfering RNA (siRNA) (2,11,12) or antisense oligonucleotides (13) against GPR40. In line with these observations, GPR40 agonists mimic the acute effect of FA on insulin secretion (14), and GPR40 antagonists block FA potentiation of GSIS (15). Importantly, potentiation of GSIS by FA is not completely abolished in the absence of GPR40, and the residual effect of FA is likely mediated by their intracellular metabolism and the generation of lipid-derived signalling molecules (Fig. 1 and reviewed in Nolan et al [16]).

In contrast to their acute, stimulating effects on insulin secretion, prolonged exposure to elevated levels of FA impairs β-cell function in the presence of high glucose, a phenomenon referred to as glucolipotoxicity (reviewed in Poitout and Robertson [17]). The possibility that GPR40 might be implicated in the mechanisms of glucolipotoxicity raised concerns that chronic administration of a GPR40 agonist could have undesirable, deleterious effects on the β-cell. In support of this possibility, Steneberg et al (10) showed that deletion of GPR40 was protective against high-fat diet-induced insulin resistance and glucose intolerance. Conversely, transgenic overexpression of GPR40 under the pancreas-duodenum homeobox-1 (PDX-1) promoter in mice led to impaired insulin secretion and hyperglycemia (10). These results led the authors to conclude that chronic activation of GPR40 is detrimental to β-cell function. Several subsequent studies, however, came to a different conclusion. First, we and others have shown that other lines of GPR40$^{-/-}$ mice are not protected from high-fat diet-induced obesity and insulin resistance (9,18). Second, Nagasumi et al (19) observed that transgenic overexpression of GPR40 under the control of the insulin II promoter in mice increases insulin secretion and protects from high-fat diet-induced glucose intolerance. Third, the impairment of GSIS (5) and induction of apoptosis (12) after chronic exposure to FA in vitro is similar in islets isolated from GPR40$^{-/-}$ and wild-type (WT) mice. Fourth, administration of a GPR40 agonist improves glucose intolerance and increases insulin secretion in rodent models of type 2 diabetes (14,20) and, most importantly, significantly lowers glycated hemoglobin (A1C) levels in type 2 diabetes patients (6). Overall, although the reasons for these discrepancies remain unknown, the majority of studies concur to suggest that GPR40 is not implicated in the mechanisms of glucolipotoxicity and that stimulating GPR40 might represent a suitable therapeutic approach in type 2 diabetes. This is supported by genetic evidence in humans, in which two loss-of-function single nucleotide polymorphisms within the *GPR40* gene were shown to be associated with impairment of insulin secretion (21).

### *GPR40 agonists as new drug for type 2 diabetes*

Based on the preclinical evidence summarized above, several pharmaceutical companies have initiated programs to develop

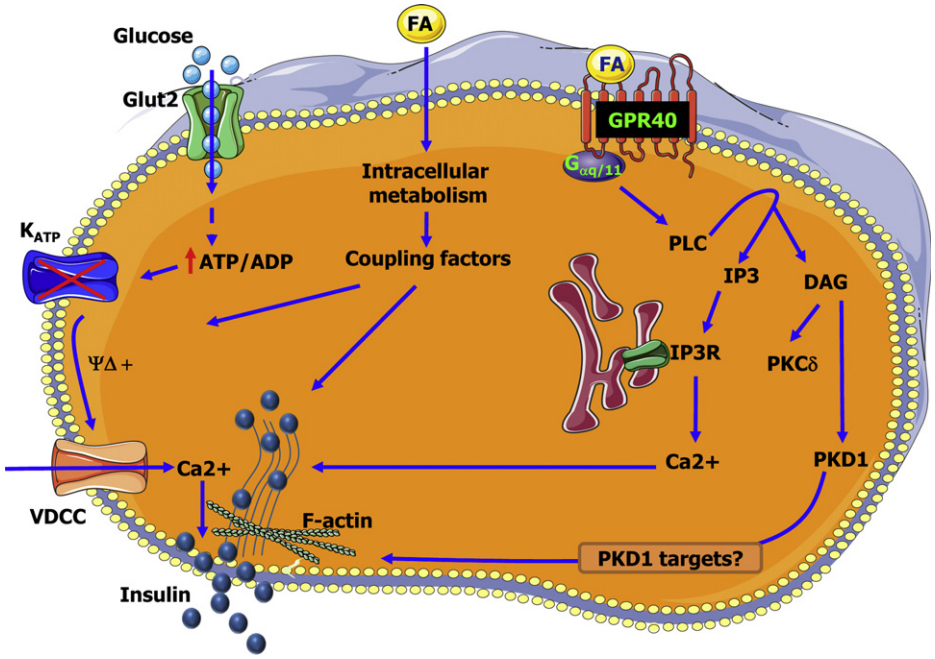


**Figure 1.** Model of GPR40-dependent and -independent potentiation of GSIS by FA.

GPR40 agonists as type 2 diabetes drugs. The only compound of this class for which, to our knowledge, the results of phase 2 trials have so far been made public is TAK-875 (6,7). In vitro activity profile showed that TAK-875 is a potent agonist ($EC_{50}$ in the nM range) with high affinity to human GPR40 (22) and high selectivity for GPR40 vs. GPR120, another long-chain FA receptor, and vs. the short-chain FA receptors GPR41 and GPR43 (22). TAK-875 potentiates GSIS in human and rodent islets, without stimulating basal insulin secretion (23,24). Although GPR40 is expressed in α-cells (25,26), TAK-875 has no effect on glucagon secretion in human islets (24). Importantly, prolonged incubation of INS832/13 cells with TAK-875 does not cause β-cell dysfunction or induction of apoptosis (23). Acute oral administration of a single dose of TAK-875 dose-dependently improves glucose intolerance and increases insulin secretion during an oral glucose tolerance test in rat models with diabetes but has no effect in normoglycemic rats (23). In humans with type 2 diabetes, a randomized, double blind, placebo-controlled trial showed significant reduction in hemoglobin A1C levels (−1.2%) after 12 weeks of treatment with TAK-875 given once daily at doses of 50 mg and higher (6). The magnitude of this A1C-lowering effect is similar to that of the sulfonylurea glimepiride but, contrary to glimepiride, is not associated with hypoglycemic episodes. Administration of TAK-875 is associated with reduction in fasting plasma glucose, improvement of glucose tolerance, and a significant increase in the insulinogenic index (a measure of β-cell function) with no changes in insulin sensitivity. Importantly, TAK-875 has no effect on blood glucose levels or insulin secretion in healthy subject (22), confirming that it is only effective when blood glucose levels are elevated. These initial results are clearly encouraging and provide an important proof of principle for a beneficial effect of GPR40 agonism on glucose control in type 2 diabetes in humans. Clearly, however, the durability of this approach and lack of long-term adverse effects remain to be demonstrated in clinical trials of longer duration.

*Mechanisms of GPR40-dependent potentiation of GSIS*

Despite the tremendous interest in GPR40 as a new type 2 diabetes target and the large volume of published studies on the pharmacology of the receptor, relatively little is known regarding its biology and, in particular, the precise mechanisms by which its activation by FA amplifies insulin secretion. GSIS is chiefly governed by the so-called triggering pathway, which involves glucose metabolism, an increase in the ATP/ADP ratio, closure of the ATP-sensitive potassium ($K_{ATP}$) channel, plasma membrane depolarization, opening of voltage-dependent calcium channels, and an increase of intracellular calcium levels ($[Ca^{2+}]_i$) that trigger insulin release (27) (Fig. 1). This triggering pathway is complemented by additional, amplifying pathways that essentially augment the secretory response to calcium influx via additional glucose-derived metabolites or membrane receptors such as the glucagon-like peptide 1 receptor, the adrenergic receptor or GPR40. However, none of these amplifying pathways is operative without the triggering pathway being activated first. Thus acutely, FA and GPR40 agonists do not initiate insulin secretion in the absence of glucose.

There are multiple mechanisms by which FA potentiate insulin secretion. A substantial corpus of literature (reviewed in Nolan et al [16]) has shown that FA can be taken up, activated and metabolized by β-cells and that signalling molecules produced by intracellular FA metabolism enhance GSIS (Fig. 1). On the other hand, the identification of GPR40 as a FA receptor suggested that FA could also regulate insulin secretion via receptor-mediated signalling. The functional contribution of GPR40-dependent FA signalling was confirmed by loss-of-function approaches in mice and insulin-secreting cells (2,4,5,8–13). Interestingly, intracellular fuel metabolism is not altered in $GPR40^{-/-}$ mice (4), suggesting that there is no cross-talk between the GPR40-dependent and metabolic pathways activated by FA (Fig. 1).

The insensitivity of GPR40 signalling to pertussis toxin (1,2), and its inability to stimulate intracellular cAMP production (2) suggest that GPR40 mainly couples to the $G_{\alpha q/11}$ subunit of heterotrimeric G-proteins, which classically catalyzes membrane phospholipid hydrolysis by phospholipase C (PLC), generation of inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG), calcium mobilization from endoplasmic reticulum (ER) stores and activation of DAG-sensitive proteins, respectively (Fig. 1). However, our understanding of the signalling mechanisms of FA via GPR40 remains incomplete, a knowledge gap that recent studies by our group and others have attempted to fill. Pharmacological inhibition of PLC dampens the increase in $[Ca^{2+}]_i$ and the potentiation of GSIS by FA in rat islets and insulin-secreting INS-1E cells (28,29). Contradictory results were observed regarding the source of $[Ca^{2+}]_i$ downstream of PLC activation. Shapiro et al (29) showed that the increase of $[Ca^{2+}]_i$ in response to palmitate is strongly reduced on inhibition of the sarco-endoplasmic reticulum $Ca^{2+}$-ATPase (SERCA) pump. In contrast, Fujiwara et al (28) reported that the increase of $[Ca^{2+}]_i$ in response to oleate is preserved in the presence of the SERCA pump inhibitor thapsigargin. Consistent with this, potentiation of GSIS by GPR40 agonists is not inhibited by blocking calcium mobilization from ryanodine-sensitive or IP3-sensitive intracellular calcium stores (30). FA also fail to augment $[Ca^{2+}]_i$ in calcium-free media (28), and decrease the amplitude of the voltage-gated $K^+$ current (31) that leads to calcium influx through voltage-dependent L-type calcium channels (LTCC) (31). Finally, opening of the $K_{ATP}$ channel by diazoxide or inhibition of LTCC abolishes the increase in $[Ca^{2+}]_i$ in response to FA (28,29). Altogether these observations suggest that the IP3 pathway likely plays a minor role in the control of $[Ca^{2+}]_i$ and insulin secretion in response to FA (Fig. 1), although contribution of IP3 signalling remains to be directly assessed. The other signalling branch downstream of PLC activation involves DAG generation and activation of DAG-responsive kinases. In a recent study, we have shown that exogenous DAG mimics the potentiating effect of FA on insulin secretion in WT and $GPR40^{-/-}$ islets (32), consistent with the possibility that DAG lies downstream of GPR40 activation in the signalling cascade. We further identified protein kinase D1 (PKD1), a DAG-sensitive kinase, to be activated and required for the potentiation of GSIS in response to FA. FA induce PKD1 phosphorylation at Ser744/Ser748 in the catalytic domain and Ser916 at the C terminus in WT, but not $GPR40^{-/-}$ islets, and pharmacological inhibition or deletion of the gene encoding PKD1 impairs the potentiation of GSIS by FA. These findings are consistent with previous studies showing the requirement of PKD1 for the potentiation of insulin secretion by agonists of the M3-muscarinic receptor, which also couples to $G_{\alpha q/11}$ (33,34). Although protein kinase C (PKC) δ can catalyze PKD1 phosphorylation at Ser744/Ser748, insulin secretion in response to FA is unaltered in $PKC\delta^{-/-}$ (32), suggesting that GPR40-dependent potentiation of insulin secretion is mediated by PKD1 activation directly by DAG or via other DAG-sensitive proteins, but not by PKCδ (Fig. 1). The contribution of other DAG-sensitive proteins such as Munc13-1 (35) in the control of insulin secretion by FA remains to be investigated.

Insulin is secreted in response to glucose in a biphasic manner. According to a generally accepted model, a readily-releasable pool of secretory granules predocked at the plasma membrane accounts for the rapid and transient first phase of insulin secretion, whereas the delayed but sustained second phase of insulin secretion requires the recruitment of granules from an intracellular reserve pool to the membrane. The second phase involves cytoskeleton reorganization and requires depolymerization of cortical filamentous (F-)actin that enables secretory granules transported along microtubules to reach the plasma membrane (36). We have

observed that FA preferentially and robustly potentiate the second phase of GSIS in mouse islets (32). This effect was associated with rapid, GPR40-dependent F-actin depolymerization, suggesting that GPR40 signalling promotes F-actin remodelling and thereby potentiates the second phase of insulin secretion. Consistent with this, PKD is known to phosphorylate proteins that regulate F-actin remodelling (37) (Fig. 1).

In summary, although recent studies have shed light on some aspects of GPR40 signalling, our understanding of the precise mechanisms by which FA binding to GPR40 potentiates GSIS remains incomplete. Several questions will need to be answered to take full advantage of this mechanism of action for therapeutic purposes and to inform the design of new type 2 diabetes drugs. Amongst those, the potential contributions of β-arrestin-dependent signalling and/or of $G_{\alpha}$ subunits other than $G_{\alpha q/11}$ remain to be examined; whether ligand-biased signalling can be harnessed for developing more efficacious and safer agonist compounds (38) remains to be tested; and more generally several important aspects of the biology of GPR40 including its potential dimerization, regulation of its internalization and desensitization remain, to our knowledge, unknown.

### *Regulation of GPR40 gene expression*

The gene encoding GPR40 is located in a cluster of 4 GPCRs (GPR40–43) on chromosome 19 in humans and is composed of 2 exons. The first small noncoding exon contributes to the regulation of GPR40 expression, and the protein is entirely encoded by the second exon (39). Characterization of the upstream promoter region identified 3 evolutionary conserved regions, HR1–3 (39). HR1 is not required for GPR40 promoter activity, whereas HR2 is required for transactivation of the gene and contains binding sites for PDX-1 and BETA2/NeuroD1, both glucose-responsive transcription factors that are important for β-cell gene expression. Mutation of the PDX-1-binding site drastically reduces transcriptional activity of the HR2 element in insulin-secreting MIN6 cells (40). The HR3 region contains the transcription start site, and a downstream promoter element (DPE) within exon1. Reporter assays revealed that HR3 lacks promoter activity and is likely required for the binding of the transcription pre-initiator complex (TFIID, RNA polymerase II) to the DPE region.

We reported that expression of GPR40 is stimulated by glucose in rodent and human islets (40). This occurs at the transcriptional level and involves glucose-stimulated binding of PDX-1, but not BETA2, to the HR2 region. We further showed that this effect of glucose is mediated by the hexosamine biosynthesis pathway (HBP) and O-GlcNAcylation of PDX1. Interestingly, O-GlcNAcylation of PDX-1 occurs at the nuclear membrane and is dependent on the formation of a complex containing PDX-1, O-GlcNAc transferase, and the product of the phosphatidylinositol-3-kinase reaction, phosphatidylinositol 3,4,5-triphosphate (40) (Fig. 2). The stimulation of GPR40 expression by glucose is accompanied by an enhancement of the potentiation of GSIS by FA.

Because PDX-1 is implicated in the regulation of GPR40 on one hand and, on the other hand, is impaired in its ability to translocate to the nucleus and transactivate its target genes in glucolipotoxic conditions (41), it is conceivable that excessive levels of glucose and FA under diabetes conditions lead to a loss GPR40 expression via defective PDX-1 function. Consistent with this possibility, GPR40 expression levels are decreased in islets from type 2 diabetes patients (42), high-fat fed mice (18) and rats infused for 3 days with glucose and lipids (43).

### *Potential roles of GPR40 in non β-cells*

GPR40 is expressed predominantly in β-cells but also in other tissues at comparatively lower levels. It should be noted however that most commercially available antibodies against rodent GPR40

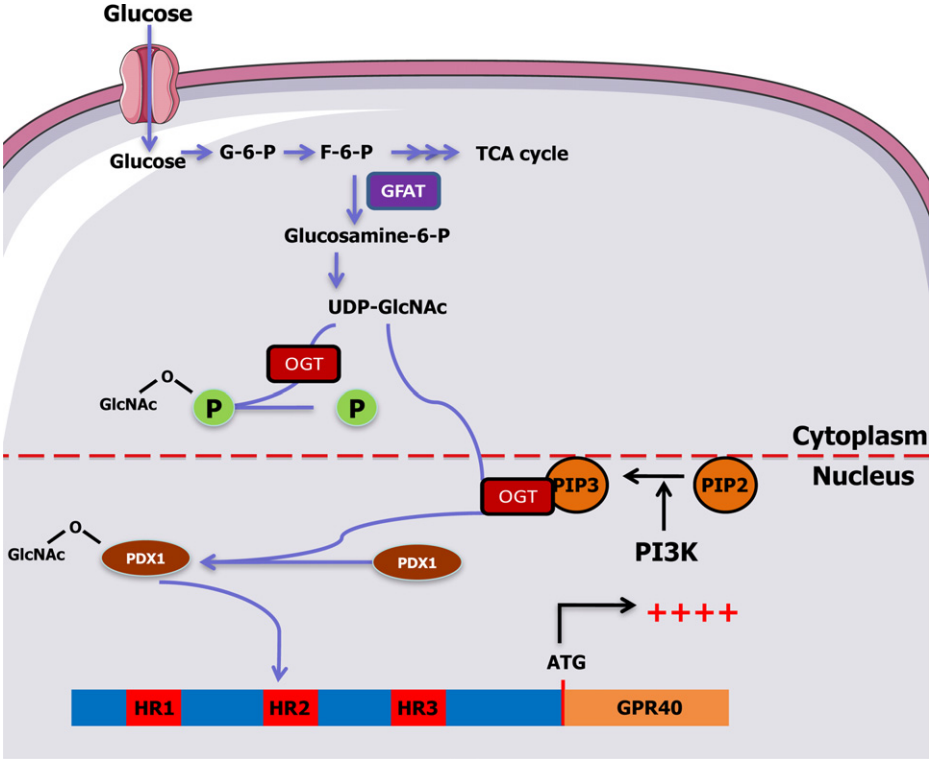


**Figure 2.** Regulation of GPR40 gene expression by glucose in pancreatic β-cells. *F6P*, D-fructose 6 phosphate; *G6P*, D-glucose 6 phosphate; *OGT*, O-GlcNAc transferase; *PIP2*, phosphatidylinositol 4,5-biphosphate; *PIP3*, phosphatidylinositol 3,4,5-triphosphate; *UDP-GlcNAc*, uridine diphosphate N-acetylglucosamine.

are of poor quality, thus limiting the comparative analysis of GPR40 protein expression levels across tissues.

Within islet, GPR40 expression in α-cells and its role in the control of glucagon secretion remains debated. Flodgren et al (25) showed that GPR40 is expressed in glucagon-producing cells as well in rat α-cells, and controls at least in part linoleic acid-mediated glucagon secretion. Wang et al (26) showed that this effect is associated with a GPR40-dependent increase in $[Ca^{2+}]_i$ downstream of PLC. In contrast, in situ hybridization in isolated rat islets showed that GPR40 mRNA is mainly expressed in insulin-positive cells (2). Finally, the GPR40 agonist TAK-875 does not stimulate glucagon secretion in rat and human islets (24), and fasting glucagon levels are unaffected by TAK-875 in type 2 diabetes patients (6).

GPR40 expression was detected in enteroendocrine cells including L-, K- and I-cells, cells producing glucagon-like peptide 1 (GLP-1), glucose-dependent insulinotropic peptide (GIP) and cholecystokinin (CCK), respectively (44,45). Interestingly, the secretion of these hormones in response to FA is impaired in $GPR40^{-/-}$ mice. These observations suggest that in addition to directly stimulating insulin secretion from β-cells, GPR40 might also enhance insulin secretion indirectly through the incretin hormones GLP-1 and GIP, although this remains to be directly demonstrated. The effect on CCK secretion suggests that GPR40 might control food intake and gastric emptying, however, these parameters are unchanged in $GPR40^{-/-}$ or transgenic mice (5,9,18,19). Interestingly, GPR40 and GPR120 are expressed in taste buds and control taste preference for FA (46).

The expression of GPR40 in the central nervous system is controversial. Although Itoh et al (2) did not detect expression in the mouse brain, in other studies GPR40 expression was observed in human, primate, rat and mouse brains (1,47). However, the functional role, if any, of GPR40 in the central nervous system remains unknown, although it has been shown to control pain regulatory systems (48).

GPR40 is also expressed in osteoclasts and appears to mediate the inhibition of osteoclastogenesis by FA (49). Consistent with these observations, our collaborator Y. Wauquier (INRA, Clermont-Ferrand, France) observed that $GPR40^{-/-}$ mice have reduced bone density. This raises the interesting possibility that GPR40 agonism, besides improving glucose metabolism, might also protect against osteoporosis. This possibility, however, remains to be formally tested.

Finally, GPR40 has been implicated in the stimulation of breast cancer cell line proliferation in response to oleate (50). This raises the possibility that chronic administration of GPR40 agonists might have undesirable effect, a possibility that obviously needs to be examined carefully.

Overall, the role of GPR40 in tissues other than the β-cell remains largely unexplored and awaits the availability of mice with tissue-specific deletion of the receptor.

## Conclusion

The discovery of GPR40 as a GPCR highly expressed in β-cells and mediating the potentiation of insulin secretion by FA prompted the development of GPR40 agonists for type 2 diabetes therapy. One of these compounds showed promising results in recent phase 2 trials, thus providing an important proof of concept for this mechanism of action in humans. As several GPR40 agonists are progressing through clinical development, there is a pressing need to better understand the basic mechanisms governing the biology of GPR40. What is the contribution of G-protein subunits other than $G_{\alpha q/11}$ and/or β-arrestin to GPR40 signalling? Do synthetic agonists differ, in their signalling properties, from the endogenous ligands? Does GPR40 internalize? What is the role of GPR40 in tissues other than the β-cell? Answering these questions will be essential to inform the development of GPR40-based drugs for type 2 diabetes, maximize their efficacy and minimize their side effects.

## Author Disclosures

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## Author Contributions

MF and VP wrote the review. MK, VB, AM and TA reviewed the manuscript.

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