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FFAR3 modulates insulin secretion and global gene expression in mouse islets

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Abbreviations: BHB, betahydroxybutyrate; DEG, differentially expressed genes; FFAR2, free fatty acid receptor 2; FFAR3, free fatty acid receptor 3; GPCR, G protein coupled receptor; GSIS, glucose stimulated insulin secretion; KRB, Kreb's ringer buffer; MCPC, 1-methylcyclopropane carboxylate; PTX, pertussis toxin; WT, wildtype.

The short chain fatty acid (SCFA) receptor (free fatty acid receptor-3; FFAR3) is expressed in pancreatic β cells; however, its role in insulin secretion is not clearly defined. Here, we examined the role of FFAR3 in insulin secretion. Using islets from global knockout FFAR3 (*Ffar3*$^{-/-}$) mice, we explored the role of FFAR3 and ligand-induced FFAR3 signaling on glucose stimulated insulin secretion. RNA sequencing was also performed to gain greater insight into the impact of FFAR3 deletion on the islet transcriptome. First exploring insulin secretion, it was determined that *Ffar3*$^{-/-}$ islets secrete more insulin in a glucose-dependent manner as compared to wildtype (WT) islets. Next, exploring its primary endogenous ligand, propionate, and a specific agonist for FFAR3, signaling by FFAR3 inhibited glucose-dependent insulin secretion, which occurred through a $G\alpha_{i/o}$ pathway. To help understand these results, transcriptome analyses by RNA-sequencing of *Ffar3*$^{-/-}$ and WT islets observed multiple genes with well-known roles in islet biology to be altered by genetic knockout of FFAR3. Our data shows that FFAR3 signaling mediates glucose stimulated insulin secretion through $G\alpha_{i/o}$ sensitive pathway. Future studies are needed to more rigorously define the role of FFAR3 by *in vivo* approaches.

Introduction

Short chain fatty acids (SCFAs) are a unique nutrient class as they originate largely from gut microbial fermentation of difficult to digest carbohydrates.[1] As the gut microbiota is a novel, recently identified, factor involved in metabolism,[1] investigating metabolic effects of SCFAs has emerged as a topical scientific question. Within this nutrient class, each SCFA is distinct and can be classified by the number of carbons in the molecule, which includes the primary SCFAs in the human body; acetate (carbon number is 2, C2), propionate (C3), and butyrate (C4).[1] While each of these SCFAs is produced at high concentrations during gut microbial fermentation, in the plasma, acetate is at the highest concentration, followed by propionate and then butyrate.[1] Recently, 2 G-protein coupled receptors (GPCRs), FFAR2 and -3 (free fatty acid receptor-2, and -3), that are activated by SCFAs have been described and observed to be expressed in multiple tissue types.[2,3] With the identification of these GPCRs, certain biological effects of SCFAs have been attributed to their signaling through these receptors.[4,5]

Of interest here, it has also been reported that these SCFA receptors, FFAR2 and FFAR3, are expressed in pancreatic β (β) cells,[6] where the primary function of β cells is the secretion of insulin to maintain euglycemia. Besides glucose, which is the primary stimulus for insulin secretion, other nutrients can act as insulin secretagogues, such as amino acids and long chain fatty acids, acting either through specific receptors or metabolic pathways.[7] As compared to these nutrients, the role of SCFAs in insulin secretion has not been well investigated.[1] Considering the existing studies, most of these studies were done over 30 to 40 years ago, and have revealed conflicting results. For acetate, some studies have observed that acetate augments[8,9] and other studies that acetate inhibits glucose stimulated insulin secretion (GSIS).[10] Compared to acetate, even fewer studies have examined propionate and butyrate in GSIS. One study observed that propionate inhibits GSIS[11] and another study observed that butyrate augments GSIS.[12] As these receptors are expressed in β cells and GPCRs have a well-described role in insulin secretion,[13] investigating if SCFAs mediate GSIS through their cognate receptors is needed. Thus far, one report observed that acetate inhibits GSIS through signaling through these receptors.[14]

As we begin to examine the role of these SCFA receptors, FFAR2 and FFAR3, in insulin secretion, the pharmacology of these receptors needs to be considered, as each of these GPCRs has unique ligand preferences and potencies for SCFAs.[15] For example, propionate is highly selective for FFAR3 as compared

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to FFAR2.[15] Also, these GPCRs signal via unique pathways.[3] Specifically, FFAR2 can signal via 2 different Gα pathways ($G\alpha_{q/11}$ or $G\alpha_{i/o}$) where signaling via each pathway is anticipated to influence GSIS differently (either augment or inhibit, respectively), whereas FFAR3 only signals via one Gα pathway ($G\alpha_{i/o}$) which is anticipated to inhibit GSIS.[13] While SCFAs can potentially affect GSIS via these receptors, SCFAs also likely can impact GSIS independent of their receptors through anaplerotic pathways as described with other nutrients.[16] Considering the above, we, here, investigated the role of FFAR3 in insulin secretion by using islets from FFAR3 ablation mice (*Ffar3*$^{-/-}$), and specific endogenous ligands and agonists/antagonists for FFAR3 to dissect specifically how FFAR3 contributes to GSIS.

Materials and Methods

Mice

Ffar3$^{+/-}$ mice (kindly provided by Dr. Yanagisawa, University of Texas Southwestern Medical Center) were maintained on a C57BL/6J background. Heterozygous *Ffar3*$^{+/-}$ mice were crossed to produce wild type (WT) and knockout (*Ffar3*$^{-/-}$) mice and genotyped by PCR as before.[17] All animal studies were conducted in accordance with regulations of the Institutional Animal Care and Use Committee at Northwestern University.

Islet isolation

Islets from male mice (age 10–14 weeks) were isolated by a collagenase (Sigma, St. Louis, MO) digestion and separated as before.[18] Isolated islets were rested overnight at 37°C in RPMI 1640 supplemented with 10% FBS, 1% L-glutamine and 1% penicillin/streptomycin (with or without 300 ng/ml pertussis toxin, PTX) prior to experiments.

In vitro insulin secretion and insulin content

For the GSIS studies, islets were selected from pooled islet isolations from 2 pancreata to ensure that sufficient islets of similar size could be selected. Next, these islets were preincubated for 30 min in Krebs-Ringer Buffer (KRB; NaCl 130 mM, KCl 4.7 mM, NaH_2PO_4 0.5 mM, $MgSO_4$ 0.5 mM, $CaCl_2$ 1.5 mM, HEPES 10 mM, BSA 0.1%, pH 7.4, where the BSA (fraction V, protease-free) was from Roche Pharmaceuticals) and then in KRB plus 2.8 mM glucose for 60 min at 37°C. Further groups of 5 islets were incubated in 1 ml KRB plus different glucose concentrations, or 16.7 mM glucose plus ligands (SCFAs, agonists, or Exendin-4, each from Sigma, St. Louis, MO) for 60 min in a shaking water bath at 37°C. Concentrations of the SCFAs were 100 μM (for propionate and butyrate) and 1 mM (for 1-methylcyclopropane carboxylate, MCPC or β hydroxybutyrate, BHB). After the last incubation period, supernatant was sampled and assayed for insulin by ELISA (ALPCO diagnostics). For islet insulin content, islets were sonicated in acid-ethanol solution and solubilized overnight at 4°C before insulin ELISA.

RNA sequencing and quantitative real time PCR

Total cellular RNA was extracted from isolated islets that were selected from pooled islet isolations from 2 pancreata (total number of islets per sample was 250) using a RNeasy Mini kit (QIAGEN). RNA-sequencing and data analyses were both carried out by the Next Generation Sequencing Core Facility at Northwestern University (n = 3 per genotype; where each sample included islets isolated from separate groups of mice). Alignment and expression analysis were performed using TopHat (v2.0.8b) and Cufflinks (v2.1.1). Differential expression was determined by cuffdiff using an FDR cutoff value of 0.05. After this, the R package, cummeRbund, was used to obtain up- and down-regulated genes. A pathway analysis was performed using GeneCoDis. The generated data is available in Gene Expression Omnibus (GEO) under submission number GSE67991. Quantitative real-time PCR was performed using 1-Step SYBR Green qRT-PCR Kit. The relative gene expression was determined by comparative ΔCt method after normalization to β actin. The primers used are available on request.

Statistical analysis

P values were determined using Student's 2-tailed t-test.

Results

Ffar3$^{-/-}$ islets secrete more insulin

As genetic knockout of FFAR3 may impact insulin secretion, as observed in studies with other GPCR knockout models,[19] we first assessed if islets from *Ffar3*$^{-/-}$ mice have altered insulin secretion to increasing concentrations of glucose. For these studies, we used islets isolated from 10–14 week old *Ffar3*$^{-/-}$ mice and age matched wildtype (WT) littermates. Both WT and *Ffar3*$^{-/-}$ islets exhibited glucose concentration dependence in insulin secretion (**Fig. 1A**). However, WT islets secreted less insulin compared to *Ffar3*$^{-/-}$ islets at each glucose concentration becoming significant at higher concentrations of glucose (**Fig. 1A**). This attenuation of GSIS from WT islets was not due to altered insulin content (**Fig. 1B**). Overall, these data suggest that genetic ablation of *Ffar3* increases the insulin secretory capacity to increasing glucose levels.

FFAR3 signaling negatively mediates insulin secretion

GSIS can be modulated by nutrients signaling via their cognate GPCRs.[7] Considering that islets express specific GPCRs for SCFAs,[6] we next assessed if SCFAs impact GSIS through FFAR3, focusing on the most potent and selective SCFAs for FFAR3, propionate and butyrate.[3] Using these ligands, we observed with WT islets, GSIS was significantly diminished by propionate by 40%, as compared to high glucose alone (**Fig. 1C**), but GSIS was not significantly altered by butyrate (**Fig. 1C**). With the *Ffar3*$^{-/-}$ islets, GSIS was not altered by propionate, suggesting that propionate inhibits GSIS through a FFAR3-dependent mechanism (**Fig. 1C**).

As nutrients can often impact GSIS through both receptor-dependent and -independent pathways,[7] we wanted to verify that

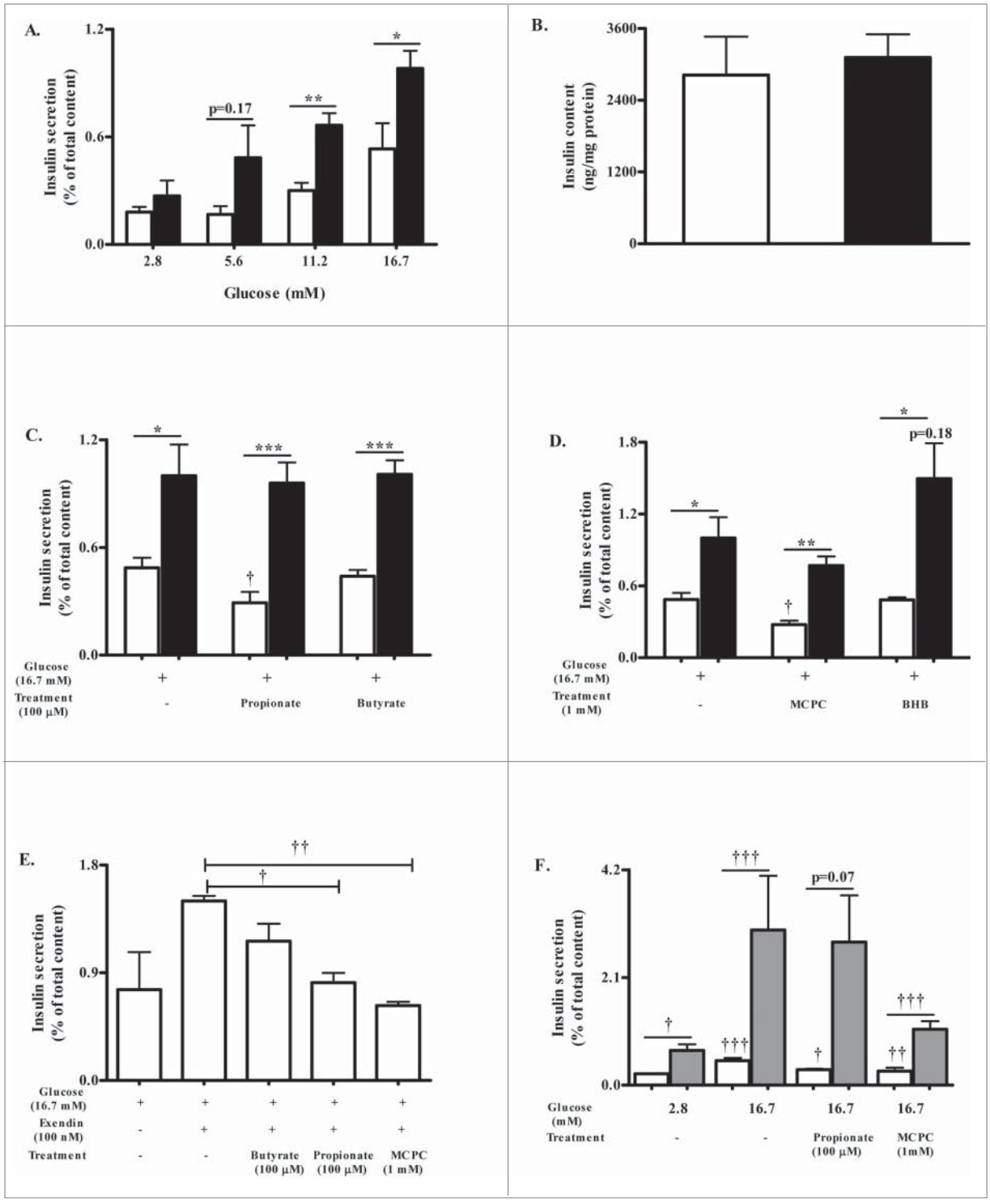

Figure 1. FFAR3 contributes to insulin secretion. (**A**) Insulin secretion in response to increasing glucose concentrations in isolated WT (white bar) and *Ffar3*$^{-/-}$ (black bar) islets during static insulin secretion assay (n $\geq$ 3). Insulin secretion is expressed as a percent of total insulin content. (**B**) Total islet insulin content measured following acid ethanol extraction from 20 islets per replicate. Insulin content was normalized to total protein (n = 3; WT, white bar and *Ffar3*$^{-/-}$, black bar). (**C**) Insulin secretion from WT (white bar) and *Ffar3*$^{-/-}$ (black bar) islets in response to treatment with high glucose (16.7 mM) alone or in combination with 100 μM propionate or butyrate (n $\geq$ 3). (**D**) Insulin secretion from WT (white bar) and *Ffar3*$^{-/-}$ (black bar) in response to treatment with high glucose (16.7 mM) alone or in combination with 1 mM MCPC or BHB (n $\geq$ 3). (**E**) Insulin secretion from WT islets in response to high glucose (16.7 mM) alone or in presence of Exendin-4 (100 nM) plus 100 μM propionate or butyrate or 1 mM MCPC (n$\geq$2). (**F**) Insulin secretion from WT islets that were pretreated overnight with PTX followed by treatment with 16.7 mM glucose or in combination with 100 μM propionate or 1 mM MCPC (n $\geq$ 3). Gray bars for PTX treated WT islets, white bars for nontreated WT islets. Asterisks represent significance between genotypes; daggers represent significance within a genotype between the indicated treatment condition compared to 16.7 mM glucose alone. *,†$P < 0.05$; **,††$P <$ 0.01; ***,†††$P < 0.001$. For A-F, mean ± SEM.

FFAR3 signaling mediates GSIS by using selective modulators for FFAR3. First, we used an agonist and antagonist of FFAR3 to explore GSIS in our model (**Fig. 1D**). The FFAR3 agonist, 1-methylcyclopropane carboxylate (MCPC), decreased GSIS with WT islets by 43% as compared to 16.7 mM glucose alone (**Fig. 1D**). This effect was not significantly present in *Ffar3*$^{-/-}$ islets, suggesting MCPC mediates its action via FFAR3. Next, using an endogenously produced FFAR3 antagonist, β hydroxybutyrate (BHB),[4] insulin secretion was not altered with WT islets and actually showed a trend toward increased insulin secretion with *Ffar3*$^{-/-}$ islets (as compared to untreated *Ffar3*$^{-/-}$ islets, $p = 0.18$).

As glucose is not the only insulin secretagogue, we next wanted to assess if FFAR3 signaling would also impact the effect of other insulin secretagogues on GSIS. Therefore, we tested whether these FFAR3 agonists affect insulin secretion in the presence of other secretagogues. Using exendin-4 (glucagon like peptide-1 receptor agonist), a well-known enhancer of GSIS, we tested whether FFAR3 ligands, propionate and MCPC, could counteract exendin-4 actions on insulin secretion in WT islets, observing that propionate and MCPC did inhibit exendin-4 induced GSIS (**Fig. 1E**).

Ligand signaling via $G\alpha_{i/o}$ mediates FFAR3 effects on insulin secretion

Determination of how FFAR3 signaling mediates GSIS is needed, where GPCRs primarily contribute to GSIS through signaling through their coupled G-proteins.[13] As reported previously, FFAR3 has been observed to couple to $G\alpha_{i/o}$ signaling pathway.[3] To determine if FFAR3 signaling via $G\alpha_{i/o}$ is the mediator of its action on insulin secretion, islets were pre-treated with pertussis toxin (PTX) to inactivate $G\alpha_{i/o}$. GSIS in PTX-treated WT islets in response to propionate or MCPC was observed to not inhibit GSIS (**Fig. 1F**), indicating that both propionate and the FFAR3 agonist mediate GSIS via FFAR3-$G\alpha_{i/o}$ signaling. However, for MCPC, there was a slight, non-significant, inhibition (comparing PTX-treated WT islets with and

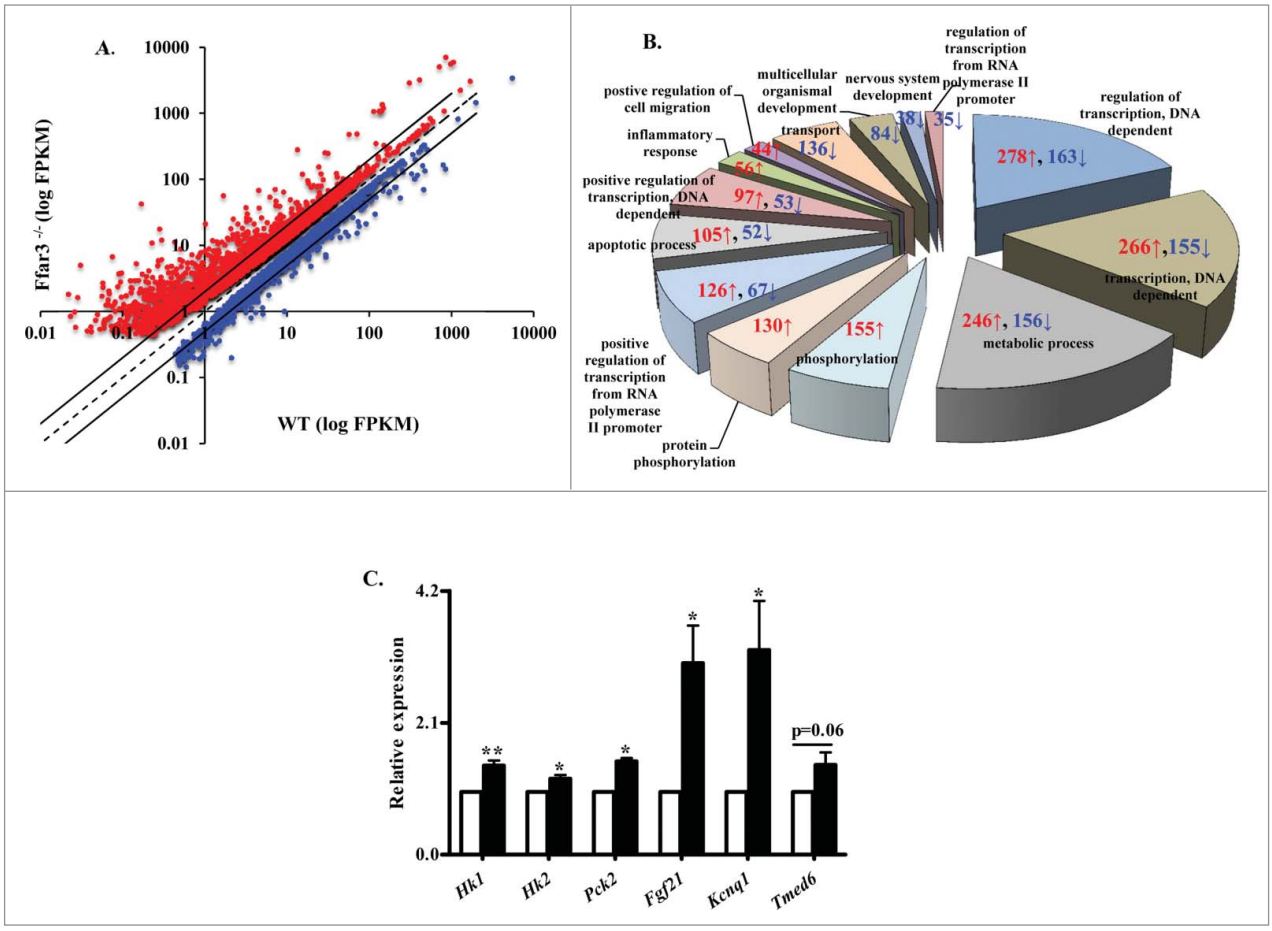

Figure 2. Genetic ablation of the FFAR3 dramatically alters the islet transcriptome. (**A**) Profile plot of the differentially expressed genes in islets isolated from *Ffar3*$^{-/-}$ mice as compared to WT mice. Red dots: genes upregulated; blue dots: genes downregulated. Transcriptome analysis was performed on RNA collected from islets that were selected from pooled islet isolations from 2 pancreata (total number of islets per sample was 250 per sample; $n = 3$ per genotype; where each sample was islets isolated from separate groups of mice). (**B**) Gene ontology chart showing biological processes enrichment obtained from analysis of differentially expressed genes in *Ffar3*$^{-/-}$ islets. Each pie chart section shows number of genes upregulated (red) and downregulated (blue) in that category. (**C**) qRT-PCR analysis of select genes from *Ffar3*$^{-/-}$ and WT islets ($n \geq 3$, 2–3 mice per group), white bars for wild type, black bars for *Ffar3*$^{-/-}$. All results are expressed as mean ± SEM. *, $P < 0.05$. FPKM- Fragments per kilobase of transcript per million mapped.

without MCPC, p = 0.1), suggesting MCPC may have FFAR3-independent effects on GSIS.

Effects of FFAR3 ablation on the islet transcriptome

To help us understand the impact of genetic ablation of FFAR3 on GSIS, we assessed the islet transcriptome in *Ffar3*$^{-/-}$ relative to WT islets. RNA sequencing was performed observing that the expression of 4165 genes was significantly altered (1626 downregulated and 2539 upregulated) in *Ffar3*$^{-/-}$ as compared to WT islets (**Fig. 2A**, see also **Table 1**). The most prominent islet markers, insulin, glucagon, glucokinase, SUR1/Kir6.2 transcripts were abundant but not altered in the *Ffar3*$^{-/-}$ islets. Interestingly, *Ghrl* and *Sst* were downregulated in the *Ffar3*$^{-/-}$ islets (both significantly) as compared to WT islets. Gene ontology (GO) analysis revealed multiple categories were affected, with metabolic, transcription and transcription regulation processes most profoundly (**Fig. 2B**). Interestingly, KEGG pathway analyses of differentially regulated genes disclosed key genes implicated in type 2 diabetes (*Tnf, Mafa, Mapk10, Pik3r3, Pik3r1, Irs2*, each were downregulated) and type 1 diabetes (*Gata4, Ptpn22, Btk*, each were upregulated).

Examining genes with known roles in islet biology, genetic alterations occurred within the insulin secretion pathway including metabolic enzymes (*Fbp2, Hk1, Hk2, Pfkfb2, Pck2*, each were upregulated), ion channels and transporters (upregulated: *Kcnq1*; downregulated: *Cacna2d2*), exocytosis machinery (upregulated: *Sycn*; downregulated: *Stxbp4*), and insulin signaling (upregulated: *Foxo1, Akt2, Eif4ebp1*; downregulated: *Irs2*). Also, expression of multiple GPCRs known to influence insulin secretion were altered (downregulated: *Ffar1, Ffar2, Glp1r, Gpr119*; upregulated: *Cckar, Ptger3*). Expression of transcription factors essential for β cell function and differentiation were also influenced by *Ffar3* ablation (downregulated: *Mafa, Pax6, Nkx6–1, Nkx2–2*; upregulated: *Foxa1, Foxa3*). Followup RT-PCR was performed, confirming the observed upregulation of *Hk1, Hk2, Pck2, Fgf21 and Kcnq1*, a key subset of genes with known roles in islet biology (**Fig. 2C**). Taken together, FFAR3 ablation results in a dramatic alteration of the islet transcriptome.

Table 1. Top 50 genes most significantly upregulated and downregulated in *Ffar3*$^{-/-}$ islets as compared wildtype islets

Upregulated	Fold Change	Downregulated	Fold Change
Sh2d1a	Inf[a]	2200002J24Rik	−3.38
Cd3e	Inf	Egr4	−3.18
Folr4	Inf	Nr4a1	−2.69
Cd3d	Inf	Fosb	−2.54
Cd19	Inf	Gpr6	−2.46
Gimap7	Inf	Dnajb1	−2.23
Banf2	Inf	Per1	−2.18
Duoxa2	Inf	Atp4a	−2.17
Ms4a1	Inf	Atf3	−2.16
Ubd	Inf	Apobec4	−2.16
Ccr6	Inf	Adamts18	−2.13
Glycam1	Inf	DXBay18,Gm14685	−2.13
Fam25c	Inf	1700045I19Rik	−2.12
Expi	Inf	Rbp7	−2.12
Timd4	Inf	Egr2	−2.08
2310057J18Rik	Inf	Arc	−2.08
Vpreb3	Inf	Ifnb1	−2.05
Fcrla	Inf	Aldh1a3	−2.04
Marco	Inf	Lrrc3b	−2.02
Icos	Inf	C2cd4a	−2.00
Igj	8.00	Nr4a2	−1.99
Prodh2	7.37	Rasd1	−1.97
Lcn2	6.51	Dnajb4	−1.97
Blk	6.40	Kcna5	−1.93
2010001M09Rik	6.00	8430408G22Rik	−1.92
Irf4	5.86	Cldn11	−1.85
Sprr1a	5.61	Hspa1b	−1.80
Faim3	5.58	Fos	−1.78
Cd2	5.40	Zfp184	−1.73
Ccr7	5.36	Irs2	−1.73
Ltb	5.35	Kcnj3	−1.72
P2ry10	5.33	3930402G23Rik	−1.71
Mpzl2	5.26	4930583H14Rik	−1.71
Il16	5.21	Tnf	−1.71
Cyp1a1	5.20	Hspa1a	−1.70
Gm5771	5.09	Egr3	−1.69
Ptpn22	4.95	Igsf21	−1.69
Stat4	4.93	Dpf3	−1.68
Il2rb	4.92	Cbx8	−1.67
Sept1	4.79	Nap1l5	−1.67
Tcf7	4.79	Cx3cr1	−1.67
Lfng	4.72	Gem	−1.65
Csf2rb	4.58	Fam167b	−1.64
Dnase1l3	4.51	Il1a	−1.64
Cd37	4.46	Kcnj2	−1.62
B3gnt7	4.45	5330411J11Rik	−1.57
Ptprcap	4.44	Edn2	−1.56
Prss3	4.42	2010110P09Rik	−1.55
Krt19	4.41	Agtr1a	−1.55
Hmgcs2	4.41	Pnmal1	−1.51

[a]Inf is abbreviated for infinity.

Discussion

A recent study by Tang et al.[14] showed that FFAR3 and a related SCFA receptor, FFAR2, are both novel β-cell expressed GPCRs contributing to GSIS. In their report,[14] the authors show that acetate, one of the 3 major SCFAs, inhibits GSIS by signaling through these receptors, and for both FFAR2 and −3, this effect on GSIS is mediated through a $G\alpha_{i/o}$-coupled pathway. Using an approach different from their report (where we used propionate and FFAR3 agonists), our data confirms their findings that FFAR3 activation inhibits GSIS through a $G\alpha_{i/o}$-coupled pathway. Thus, in conjunction with this report from Tang et al.,[14] it is apparent that FFAR3 is a novel mediator of insulin secretion.

An important difference between these 2 studies is that our data indicates that knockout of FFAR3 leads to increased insulin secretion in response to increasing glucose levels. Multiple reasons could explain this difference. First, Tang et al.[14] did not explore GSIS at multiple glucose concentrations, as done here. Another possible explanation is that the genetic approach used to create these FFAR3 knockout mouse models was different, and as a consequence, the downstream genetic changes could be unique between each model. Considering the degree of changes

in our transcriptome analyses, this seems plausible. Other possibilities are that the experimental conditions used to assess insulin secretion were not the same and/or the backgrounds of the mouse models were different. Regardless, other GPCR knockout models have shown to have alterations in *in vitro* GSIS,[19] but not all,[20] and our particular FFAR3 knockout model has enhanced GSIS with increasing glucose concentrations, as compared to WT islets. Whether or not the change in insulin secretion in response to glucose is the result of FFAR3 deletion itself or other factors is not clear at this time.

A few other notable findings regarding our study warrant discussion. First, activation of FFAR3 resulted in significant inhibition of GSIS by either propionate or the FFAR3 agonist; however, this effect size is modest (see **Fig. 1C–D**). A possible reason for this modest inhibition of GSIS is that FFAR3 has been suggested to have high ligand independent constitutive activity.[15] Thus, activating a constitutively active GPCR would only result in a small change in receptor activity. Unfortunately, for our understanding of this GPCR, this high constitutive active of FFAR3 may be specific to mouse FFAR3 and not human FFAR3.[15] Therefore, as future studies explore the role of FFAR3 in human islets, close attention to species differences in receptor pharmacology such as constitutive activity needs to be considered.

Next, propionate, but not butyrate, was observed to inhibit GSIS. In the first published reports on these receptors, it was noted that propionate and butyrate were more specific to FFAR3 (as compared to FFAR2);[3] however, these observations were determined using human FFAR2 and FFAR3. Since these original reports, it has been observed that mouse and human orthologs of each receptor have distinct pharmacology for SCFAs. For example, propionate is 12 times more potent at activating mouse FFAR3 than the other endogenous SCFAs,[15] whereas butyrate is equally potent at FFAR3 and the other SCFA GPCR, FFAR2. However, it is not clear why butyrate does not inhibit GSIS in our study as FFAR2 signaling has been reported to also inhibit GSIS, as reported by Tang.[14] Taken together, our data demonstrates that propionate inhibits GSIS, an observation consistent with previously published findings.[11] Future studies need to consider species difference in ligand preference especially when exploring the role of FFAR3 in human versus mouse islets.

Lastly, BHB has been suggested to be a FFAR3 antagonist,[4] and therefore, we utilized it here to explore FFAR3 signaling. However, we did not observe an increase in GSIS from BHB, as we hypothesized. Subsequent studies have actually suggested BHB is not an antagonist, but an agonist for FFAR3,[21] and we did not observe this possibility either (for example, decreased GSIS). Considering these 2 studies and our findings, it is unclear if BHB signals through FFAR3 in mouse islets. Of interest, most reports suggest that BHB alone has little effect on GSIS,[22,23] which is consistent with our data.

The islet transcriptome analyses performed here revealed widespread genetic changes in the *Ffar3*$^{-/-}$ islets compared to WT islets. Moreover, many genes that specifically contribute to GSIS were either up or downregulated. Whether or not this leads to meaningful changes in GSIS is unclear, but it could possibly explain the increased glucose responsiveness of the *Ffar3*$^{-/-}$ islets. Two genes, in particular, that were upregulated, *Hk1* and *Hk2* (which correspond to hexokinase 1 and 2), are hexokinases with low Km values for glucose and if overexpressed would result in more robust insulin secretion, as observed before.[24,25] While genetic GPCR knockout models have been observed to impact the expression of other genes and in particular genes important in islet biology,[26,27] the impact of these additional genetic changes on functional outcomes such as GSIS needs to be considered.

Taken together, our results, along with recent findings,[14] establish FFAR3 as a negative GSIS modulator through its signaling by a $G\alpha_{i/o}$ pathway. As GPCRs are important diabetes targets,[13] consideration of FFAR3 antagonists as a novel mechanism to enhance GSIS and as a viable diabetes treatment approach is needed.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed

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