Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at *Biomedicines* **2020**, *8*, 154; doi:10.3390/biomedicines8060154

Review Article

FFAR2/3 as Microbial Metabolite Sensors to Shape Host Health: Pharmacophysiological View

Sidharth P Mishra[1,2], Prashantha Karunakar[3], Subhash Taraphder[2] and Hariom Yadav[1,4,*]

[1] Department of Internal Medicine, Molecular Medicine, Wake Forest School of Medicine, Winston-Salem, NC, USA
[2] Department of Animal Genetics and Breeding, West Bengal University of Animal and Fishery Science, Kolkata, West-Bengal, India
[3] Department of Biotechnology, PES University, Bangalore, Karnataka, India
[4] Department of Microbiology and Immunology, Molecular Medicine, Wake Forest School of Medicine, Winston-Salem, NC, USA

* Correspondence: hyadav@wakehealth.edu

Abstract: Role of gut microbiome in human health is becoming apparent. The major functional impact of gut microbiome is transmitted through the microbial metabolites that are produced in the gut and interact with host cells either in the local gut environment or get absorbed in the circulation to impact distant cells/organs. Short chain fatty acids (SCFAs) are the major microbial metabolites that are produced in the gut through fermentation of non-digestible fibers. SCFAs are known to function through various mechanism, however, their signaling through free-fatty acid receptor 2 and 3 (FFAR2/3; type of G-coupled protein receptors) is new therapeutic approach. FFAR2/3 are widely expression in diverse cell types in human and mice, and functions as sensors of SCFAs to change several physiological and cellular functions. FFAR2/3 modulates neurological signaling, energy metabolism, intestinal cellular homeostasis, immune response and hormone synthesis. FFAR2/3 functions through Gi and/or Gq signaling, that is mediated through specific structural features of SCFAs-FFAR2/3 bindings and modulating specific signaling pathway. In this review, we discussed the wide-spread expression and structural homologies between human and mice FFAR2/3, and their role in different human health conditions. This information can unlock opportunities to weigh the potential of FFAR2/3 as drug target to prevent human diseases.

Keywords: FFAR2; FFAR3; microbiota; gut; immune; SCFA

1. Introduction

Gut microbiome and its contribution in human health is an emerging area and lot remains to learn about the interactions between microbial cells with host. The major communication between microbiome and host cells take place through the metabolites produced by gut microbiome [1]. These metabolites are sensed by host cells through various mechanisms. The major class of microbial metabolites are short chain fatty acids (SCFAs; like acetate, propionate and butyrate) that are either utilized by intestinal cells and/or get absorbed to enter into the circulation [2-9]. One of the major pathway by which SCFAs functions on target cells is by activating free fatty acid receptor 2 and 3 (FFAR2/3), that are type of G-coupled protein receptors (GPCRs) [2-4]. FFAR2/3 are abundantly

© 2020 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

expressed on intestinal cells and other cell types in the host and known to regulate various physiological and cellular functions. Both the receptors differentially expressed in intestine [2,10-12], adipose tissues [13,14], pancreas [15], bone marrow [9], liver [16,17], muscle [18,19], spleen [20,21], lungs [5,20,21], heart [22] and brain [16,23]. Wide spread expression of FFAR2/3 make them to play an important role in several human diseases such as type-1 and -2 diabetes [10,24-28], obesity [25,29-32], inflammatory bowel disease (IBD) [33,34], Crohn`s disease [35], cardiovascular diseases [19,36,37], gout [38], asthma [5,39,40], arthritis [34], colitis [34,41-44].

FFAR2/3 are the cell surface receptors that can play a very significant role in intracellular cell signaling [45,46]. Both FFAR2/3 receptors activate the heterotrimeric G-coupled protein intracellularly, by binding with endogenous SCFAs at the cell surface of any specific tissues. Both FFAR2 and FFAR3 are characterized as seven transmembrane (7TM) spanning proteins and consist of ~2% of whole human and mice genome sequence respectively [45,47,48], and are coupled with $G\alpha_{i/o}$ coupled signaling [21,49] and only FFAR2 is associated with $G\alpha_{q/11}$ coupled signaling [31,50]. Therefore, based on expression pattern, structural importance and activation of FFAR2/3 receptors by gut microbiota metabolites at different tissue level provides a new scope to investigate the importance of these receptors on human diseases. However, the source of information about detailed expression patterns, structural and functional analyses, and their biological functions in different human diseases and health conditions is obscure.

2. Experimental Section

Here we performed an extensive search of the literature and compiled the detailed information about the expression (gene and protein) patterns and comparative structural analysis developed by using our own insilico designed model setup of FFAR2/3 receptor. We described the expression information from literature as well as also from our cell line repository. In addition, we have performed literature search using PubMed, Google Scholar and Web of Knowledge using different combinations of key words FFAR2, FFAR3, GPR43, GPR41, microbiota, short chain fatty acids, diet, fibers, human health, diabetes, obesity, colon, intestine, adipose tissue, liver, lungs, disease, metabolic disease, stem cell, monocyte, Colitis, expression, structure, modeling, mice, knockout, human, agonist, antagonist, activator, inhibitor, software, cell line, enteroendocrine, ligand, docking, tight junction, inflammation, mucus, brain, neuron, organoid with diverse combinations.

Protein sequences and structural information was obtained from protein data base site and modeled by using modeller software and I-TASSER to develop the models for further docking with ligands, also comparing and verifying with specific ligands in human and mice. This article will provide one stop detailed and most current information about the FFAR2/3 expression, structure and their biological roles.

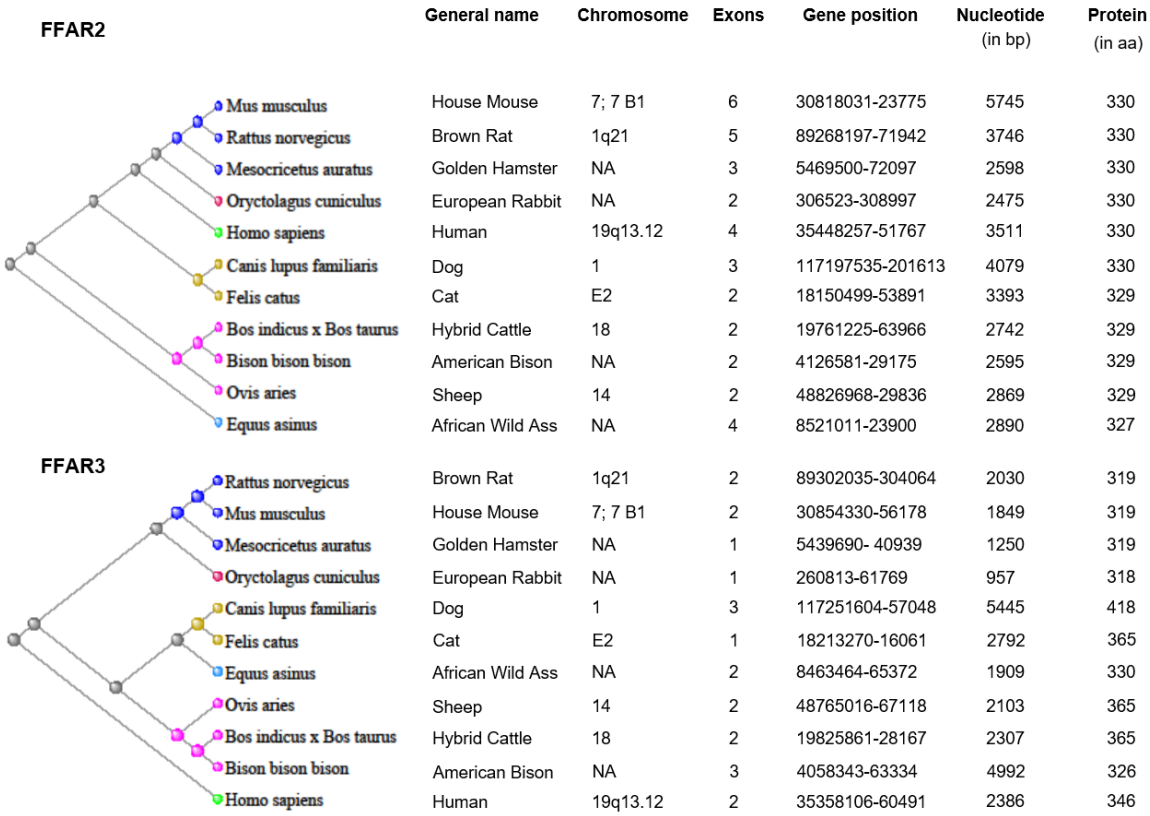
2. Expression of FFAR2 and FFAR3 in different species and tissues/cells

FFAR2 and FFAR3 are present on close proximity on chromosome 1 (19q13.12) in human and Chromosome 7 (7; 7 B1) in mice. The phylogenetic tree and detail information on FFAR2/3 chromosomal location, gene length and position, number of exons and protein length are shown in **Figure 1**. FFAR2/3 are widely expressed in different human and mice tissues, cells and cell lines, and

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

so the other species too. Below, we summarized the updated information about the expression of FFAR2/3 in humans, rodents and other species, along with their cell types.



	General name	Chromosome	Exons	Gene position	Nucleotide (in bp)	Protein (in aa)
Mus musculus	House Mouse	7; 7 B1	6	30818031-23775	5745	330
Rattus norvegicus	Brown Rat	1q21	5	89268197-71942	3746	330
Mesocricetus auratus	Golden Hamster	NA	3	5469500-72097	2598	330
Oryctolagus cuniculus	European Rabbit	NA	2	306523-308997	2475	330
Homo sapiens	Human	19q13.12	4	35448257-51767	3511	330
Canis lupus familiaris	Dog	1	3	117197535-201613	4079	330
Felis catus	Cat	E2	2	18150499-53891	3393	329
Bos indicus x Bos taurus	Hybrid Cattle	18	2	19761225-63966	2742	329
Bison bison bison	American Bison	NA	2	4126581-29175	2595	329
Ovis aries	Sheep	14	2	48826968-29836	2869	329
Equus asinus	African Wild Ass	NA	4	8521011-23900	2890	327
Rattus norvegicus	Brown Rat	1q21	2	89302035-304064	2030	319
Mus musculus	House Mouse	7; 7 B1	2	30854330-56178	1849	319
Mesocricetus auratus	Golden Hamster	NA	1	5439690- 40939	1250	319
Oryctolagus cuniculus	European Rabbit	NA	1	260813-61769	957	318
Canis lupus familiaris	Dog	1	3	117251604-57048	5445	418
Felis catus	Cat	E2	1	18213270-16061	2792	365
Equus asinus	African Wild Ass	NA	2	8463464-65372	1909	330
Ovis aries	Sheep	14	2	48765016-67118	2103	365
Bos indicus x Bos taurus	Hybrid Cattle	18	2	19825861-28167	2307	365
Bison bison bison	American Bison	NA	3	4058343-63334	4992	326
Homo sapiens	Human	19q13.12	2	35358106-60491	2386	346

Figure 1. Phylogenetic tree and genomic location of FFAR2/3

2.1 FFAR2

During the initial days of FFAR2 discovery, it was found to be intensively express in human immune cells such as peripheral blood mononuclear cells (PBMC) and polymorphonuclear cells (PMNs) with maximum expression in neutrophils [51-55]. However, more recent studies and our own data show that FFAR2 is expressed in human fungiform taste buds [56], dendritic cells (DCs) derived from bone marrow [57], liver [53], heart [53], pancreatic islet of Langerhans [58,59], spleen [53], fetal membranes and placenta [60], L-cells in large intestine [34,61], brain parenchyma [62], neuronal cell line- SK-N-SH, and human breast cancer cell line (MCF-7) [63]. Also, FFAR2 expressed in colonic epithelia and mucosa but not in the colonic muscle and submucosal regions [64]. Its expression in muscle remains controversial [53,65]. Although, FFAR2 expression is seen in brain parenchyma, but comprehensive studies on its expression in brain are needed to define its importance in brain functions [62].

FFAR2 expresses in hypothalamus [18], bone marrow [9,57], heart [66], liver [3,4,18], PBMC [34,51-54,67], stomach [18], pancreatic islet β-cells [24], lungs [39], epidermal fat pads [3,18], white adipose tissue (WAT) including perirenal, epidermal and subcutaneous tissues [21,25], ileum and colon [3,18] particularly intestinal epithelial cells (IECs) [27], L-cells [10], I-cells [2], K-cells [2] and Myeloid (M)-cells [68,69], cecum [70], and muscles [18] of mice. FFAR2 also express in the mice pancreatic β-cell line MIN6 [58,59,71,72] and adipocyte cell line 3T3-L1 [18,29,30,73,74]. In rats, FFAR2

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

found to be expressed in enteroendocrine cells and mucosal mast cells of rat and mice [34,75,76]. In both mice and rat, FFAR2 express only in epithelial and mucosal layer but not in muscle and submucosal region of colon [18,64,77]. Expression of FFAR2 in central nervous system [76] and brown adipose tissue (BAT) [29,32] is unclear, and need to be further studied comprehensively.

FFAR2 expresses in hypothalamus, cerebral cortex [78], pituitary gland [78], heart [78], liver [78,79], pancreas [78], rumen [78] particularly rumen papillae [80], omasum [78], reticulum [78], spleen [78], pancreas [78], kidney [78], adrenal gland [78], colon [78], adipose tissues [81,82] and skeletal muscle [78,82] of bovine. FFAR2 expression on bovine adipose tissue and testis is still debatable [11,78,83]. It is also expresses in bovine mammary epithelial cell line (bMEC) [84]. In swine, FFAR2 expresses in heart [12], liver [12], spleen [12,85], pancreas [85], adipose tissues [12,85,86], kidney [12], small intestine [87], caecum [87], colon [87], skeletal muscle [12,88]. In sheep, FFAR2 expresses in abomasal (distal gastric) lymph nodes [89]. In New Zealand Rabbits FFAR2 expresses in thymus, spleen, pancreas, adipose tissue, lungs, duodenum, jejunum, cecum ileum and colon [90]. In chicken, FFAR2 paralog genes are expressed in testis, spleen, blood PMBCs, adipose tissues, intestine, lung, liver, pancreas, ovary, thigh muscle, pectoralis muscle, eye, skin, sub-cutaneous adipose tissues, kidney, brain, heart, uropygial gland [91]. Feline and canine soft tissue sarcoma as well as normal skin of feline and skeletal muscle of canine also showed the FFAR2 expression [92]. FFAR2 is also found to be expressed in horse placenta [93]. However, FFAR2 expression on other experimental models are not reported till dated.

2.2 FFAR3

FFAR3 expression is detected in central nervous system (CNS) [51,94], brain endothelium [62], sympathetic nervous system (SNS) [22,62,95], bone marrow [51,52], liver [65], spleen [51,52], pancreatic β-cells [71,96,97], WAT [51,52,98], fetal membrane along with the mother placenta [60], small and large intestine [51,65,94], skeletal muscles [65], lymph node [51], and immune cells [40,51,52] of humans. It is also expressed in human breast cancerous cell line like MCF-7 [99], colorectal cancer (CRC) cell line (HCT116) [100] and human embryonic kidney cell line, HEK293 [101].

In rodents, FFAR3 expression is detected in superior cervical ganglia (SCG) and celiac sympathetic-mesenteric ganglia (CSMG) of autonomic nervous system selected randomly from different tissue region [22,95,102,103], PNS like enteric nervous system and sensory neurons [2,22,104,105], hypothalamic region of brain [18], heart [66], liver [4,18], stomach [18], pancreatic β-cells [71,96,97], adipose tissues [98], intestine (ileum and colon) [18,65,106] in the IECs [27,107], tuft cells [108-110], neuropeptide precursors and neurotensin (NeurogD3 and Neurogenin3) enteroendocrine cells like L-cells [10], I-cells [2], K-cells [2] and enteric neurons [2] and colonic mucosa [77] and skeletal muscles [65]. It is also expressed in mouse cell lines like Hepa1-6 [18,65], 3T3-L1 [51,73,74,111], 3T3-F442A [51], Ob-Luc [98] and myotubes, C2C12 [111].

FFAR2 expression is detected in bovine cerebral cortex [78], hypothalamus [78], pituitary gland [78], heart [78], lungs [78], liver [78], pancreas [78], spleen [78], rumen [78,80], kidney [78], adrenal gland [78], adipose tissue [11,81,83], duodenum [78], colon [78], skeletal muscle [78] and bMEC cell line [84]. In swine, FFAR3 expresses in heart [12], kidney [12], liver [12], adipose tissue [85], spleen [12,85], gastrointestinal (GI) tract [87] and skeletal muscle [12]. But still FFAR3 expression in swine adipose tissues is debatable [12,85]. FFAR3 gene expression was found in the colon, cecum, thymus,

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

spleen, pancreas, adipose tissue, lungs, duodenum, jejunum, ileum of New Zealand Rabbit [90]. FFAR3 gene reported to be absent in chicken genome [91]. FFAR3 was also found to express in adipose tissues of goat [112] and sheep [113].

Comprehensive expression analysis of FFAR2 and FFAR3 at different tissues and cell lines of mice and human are presented in **Table 1 and Figure 2**.

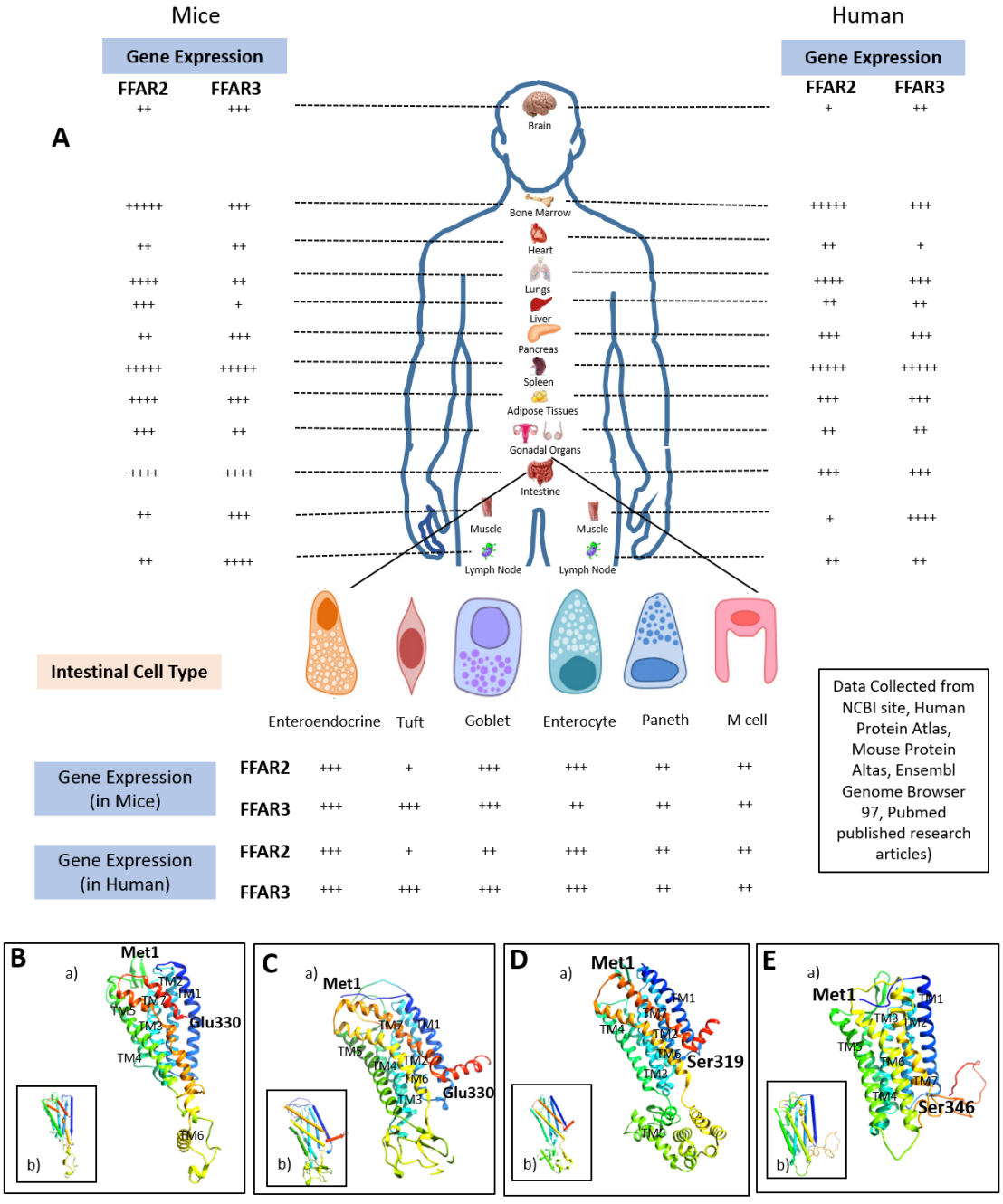


Figure 2. A) Diagrammatic representation of FFAR2 and FFAR3 expression in human tissues/cells and their comparison with mouse tissues/cells. B-E) Homolgy structure of mouse (B,D) and human (C,E) FFAR2 (A,B) and pFFAR3 (D,E) protein. a,b) Depicts the rainbow (a) and pipes and plank (b) structures.

3. Structures of FFAR2 and FFAR3

Multiple emerging evidence indicate that FFAR2/3 can be a novel target to prevent/treat several human diseases [38,58,114-118]. However, lack of knowledge in their structure and precise

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

understanding of their interactions with ligands, lead to delay in gaining attention for years to considered as a novel therapeutic targets. However, growing understanding using evolved computational *in-silico* analyses and their significant role in several human diseases such as obesity, diabetes, IBD and aging, FFAR2/3 are emerging as a potential therapeutic target [119-122]. Homology modelling of FFAR2 and FFAR3 along with gene mutagenesis, structural conformation and protein-ligand interaction are developed and their importance are discussed in the following section.

3.1. FFAR2.

Crystal structure of FFAR2 was predicted based on human β_2-adrenergic receptor [123]. Human FFAR2 comprises 330 amino acids (AAs) that are arranged in a 7-TM structure [119,124]. Structurally, the third TM of FFAR2 contains cysteine residue at the top and an arginine residue at the bottom [101,120], and a conserved domain of GPCR family- Glu-Arg-Tyr motif [101]. The active site of human FFAR2 is consists of Tyr^{90}, Ile^{145}, Arg^{180}, Arg^{255}, and Glu^{166} [45,125]. The small carboxylic acids (SCAs) binds to this binding pocket of Tyr^{90}, Ile^{145} and Glu^{166} [125]. Arg^{180} and Arg^{255} are positively charged orthosteric site which interact with negatively charged glutamine residue (Glu^{171}) to stabilize the protein structure for proper binding with ligands [45].

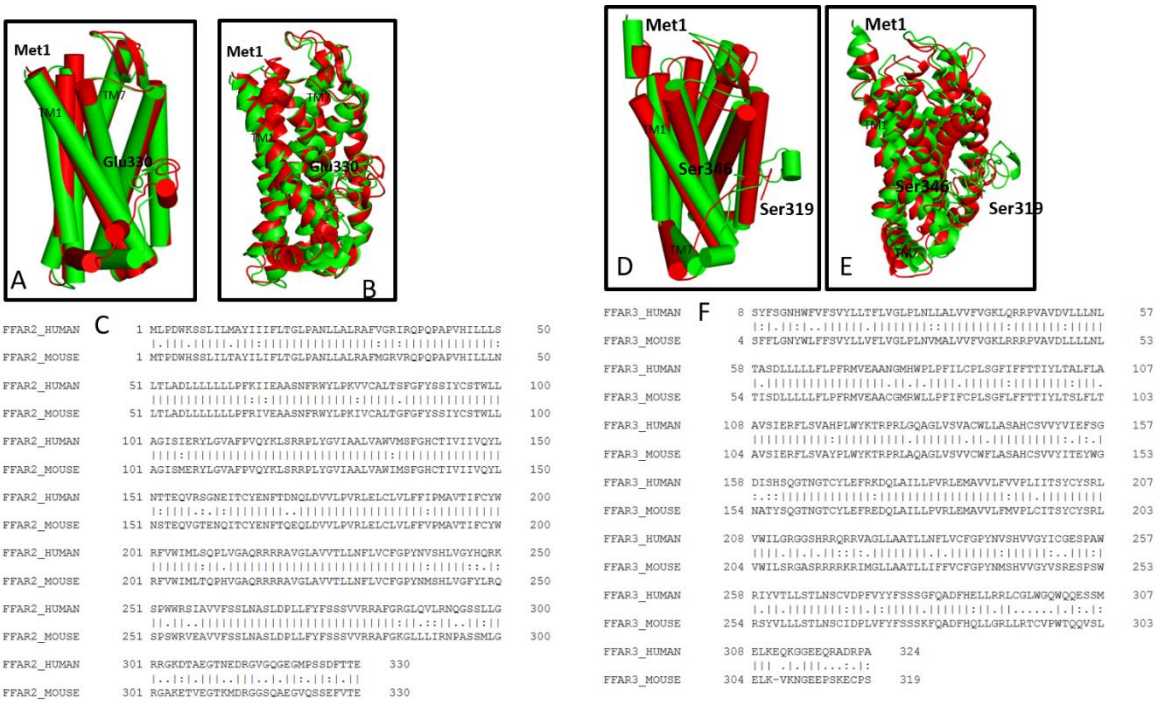


Fig. 3. Superposition of mice and human FFAR2 and FFAR3 receptors A-F) Pipes and Plank model (A,D); Rainbow Ribbon Model (B,E); and RMSD pairwise alignments of mouse and human FFAR2 (A-C) and FFAR3 (D-F) receptor sequence.

Similar to human, mouse FFAR2 is also a 7-TM protein made of 330 AAs [124], and shows 81.69% (nucleotide level) and 84.85% (protein level) similarity with it. The mice FFAR2 receptor sequence superposition and pairwise alignment with human FFAR2 is shown in **Figure 3.1**. The active binding site of mice FFAR2 comprises of Trp^{75}, Gln^{148}, Tyr^{238}, Arg^{65}, Arg^{180}, Tyr^{90} and Arg^{255} according to our insilico analysis. Both Mice and Human FFAR2 shows the protein sequential similarity from the 95-111 AAs position except at the 105^{th} position where mouse FFAR2 consists of methionine whereas human FFAR2 is of isoleucine. The significant change in the secondary structure of human and mice FFAR2 is observed at the C-terminal end. The C-terminal of a receptors consists of most conserved

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

minimotifs and short peptides that regulate receptor binding efficiency, posttranslational modification and trafficking with unique biochemical and physiological property [126]. The detail information on C-termini biophysical properties of FFAR2/3 are out of context and yet to be study in more comprehensive manner.

The FFAR2 like protein in chicken contains 367 AAs, encoded with gene of 1105 bp nucleotides as per NCBI latest update. The chicken FFAR2 paralog homology model shows four active AA residues at the position His^{140}, Arg^{180}, His^{242} and Arg^{255} [91]. These residues are further supported by Thr^{201}, Glu^{113} and His^{115}, and associated with protein-ligand interactions [91]. The homology models and chromosomal location of other laboratory animal species (*Mesocricetus auratus*, *Cavia porcellus* and *Oryctolagus cuniculus*) are not yet known and studies are needed to comprehend their location, structure and functionalities.

3.2. FFAR3

Human FFAR3 structure was also predicted based on the crystal structure of the human β_2-adrenergic receptor [123]. Human FFAR3 made of 346 AAs, and has 52% of AA sequence similarity with mice FFAR2 [119,124]. Like FFAR2, FFAR3 also contain an Arginine at the bottom of third TM domain [101,120] which contains Glu-Arg-Phe motif of GPCR family class A [101]. AA residues- Phe^{96}, Tyr^{151} and Leu^{171} involve in specific ligand binding [125] with SCAs including SCFAs. The presence of positively charged Leu^{171} residues provide stabilization to negatively charged Arginine residues at second extracellular loop (EL2) [45]. Polymorphism of FFAR3 can be considered for detail comprehensive genetic and pharmacophysiological study against various diseases [95,127].

Mouse FFAR3 is 319 AAs long [124] and has similarity of 80.41% (nucleotide level) and 76.66% (protein level) with human FFAR3. Our, insilico analysis between human and mouse FFAR3 proteins showed two substitutions at A103S and A107T site, and significant differences in human and mouse FFAR3 secondary structure was found at the C-terminal end. A superimposed structure and pairwise alignment of human and mice FFAR3 is shown in **Figure 3.2.** Detail information on chromosomal location and structural analyses of FFAR3 protein from other species are not available, and need further comprehensive studies.

3.3. Comparative structural analyses of FFAR2 and FFAR3

The structure activity relationship (SAR) study showed that the endogenous binding site volume of human homology FFAR3 (105 $Å^3$) is twice more than the volume of FFAR2 (41 $Å^3$) [128]. SAR helps in determining the chemical structure of a receptor, its relationship with the chemical compounds associated with any biological activity and chemical structural modification in the receptors to increase the biological activity of the compound [129]. Along with SAR, solvent accessible surface area (SASA) also helps in determining the molecular interaction of a biomolecule with the surrounded solvent to judge its biological effect on the organism [130]. SASA analysis of human and mice FFAR2/3 revealed that both have significantly higher hydrophobic residues than hydrophilic residues [125]. Human FFAR2 receptor have higher SASA hydrophobicity by 39 $Å^2$ and higher aromatic SASA value by 63 $Å^2$ as compared to human FFAR3 [125]. Based on virtual docking of different allosteric compounds to these receptors, it was found that FFAR2 pockets has larger volume (553 $Å^3$) and surface area (510 $Å^2$) as compared to FFAR3 binding pockets [119]. However, the volume

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

of FFAR3 binding cavity (385 Å^3) is larger than FFAR2 (332 Å^3) [125] whereas the depth of FFAR2 pockets are less than FFAR3 pockets by 2 Å [119]. So, focus must be given on the compounds having diverse small part SCAs (having lipophilic tail like branched, cyclic and unsaturated structure) for proper binding with FFAR2/3. According to Tikhonova et al. [125] prediction the subtype preferably selective binding residues between FFAR2/3 are Tyr^{90}, Ile^{145} and Glu^{166} in FFAR2 and Phe^{96}, Tyr^{151} and Leu^{171} in FFAR3. Thus, SAR information shows that the FFAR2 and FFAR3 are lying very close proximity with each other and can interact with same chemical compound to compensate each other's biological response [100]. So, more intensive and precise study must be done to determine the individual biological function of each receptor and it's binding to a particular ligand.

4. Interactions of SCFAs with FFAR2/3

4.1. FFAR2

SCFAs are orthosteric ligand of FFAR2/3 as they bind to endogenous binding sites [128]. SCFAs carboxylic group interact with the arginine groups of third, fifth and/or sixth TM domain of FFAR2 for efficient binding [128]. This SAR data explained that FFAR2 prefers flat unsaturated moieties within the SCAs [128]. So, FFAR2 mostly bind to the ligands with sp^2- or sp-hybridized α-carbon [128]. That means carbon atoms of SCAs form covalent bond with either two or one Hydrogen (H) atom for interaction with FFAR2. This concept has been further justified by Tikhonova and Poerio [125] by showing that the FFAR2-selective binding with Tiglic acid as a orthosteric ligand (binding of the ligand at endogenous site) forms a network intensive H-bonds, while leaving a small binding cavity in FFAR2. However, substitutional mutation of histidine at His^{140} and His^{242} residues to alanine in the fourth and sixth TM domain decrease the binding potential of SCFAs to FFAR2 [131,132]. Using site specific mutagenesis revealed that arginine (Arg^{180}, Arg^{255}) mutation at the top of either five and/or seven TM helix are important for facilitating the interactions of SCFAs with human and mice FFAR2 [101] (**Figure 4**). Through FFAR2 signaling, acetate moved to the peripheral tissue to regulate lipogenesis, cholesterol metabolism and control central appetite [133,134]. Moreover, propionate is responsible in maintaining whole body energy metabolism by controlling satiety signaling via FFAR2 [16,23]. Activated FFAR2 signaling by propionate treatment to human breast mesenchymal-like MDA-MB-231 and MDA-MB-436 cell inhibited Hippo-Yap pathway to reduce metastatic [135]. In addition FFAR2 signaling mediated by butyrate treatment to human enteroendocrine cell lines such as, NCI-H716 (colorectal cell line) and HuTu-80 (duodenal cell line) increases (Peptide YY) PYY gut hormonal synthesis [136].

So far, no insilico study demonstrated the SCFAs binding site with mouse FFAR2 receptor. Recently, we determined the common active binding site residues (n=31) of mouse FFAR2 through homology modeling (Un-published data). The mice FFAR2 interacts with SCFAs by forming the H-bonds, such as acetate make H-bonds with Tyr^{90}, Gln^{148}, Trp^{75} and Arg^{65}; propionate makes H-bonds with Tyr^{90}, Ile^{145}, Arg^{255}, Tyr^{238}, Arg^{180} and His^{242}; and butyrate makes H-binds with Trp^{75}, Gln^{148}, Tyr^{238}, Arg^{65}, Arg^{180}, Tyr^{90}, Arg^{255}. Similar to human, the mice FFAR2 activated by acetate and butyrate by making H-bonds with Arg^{180}, Tyr^{190} and His^{242} residual sites [25,136]. In mice also, activated FFAR2 receptor regulates the biological functions such as hormonal synthesis [136], systemic inflammation

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

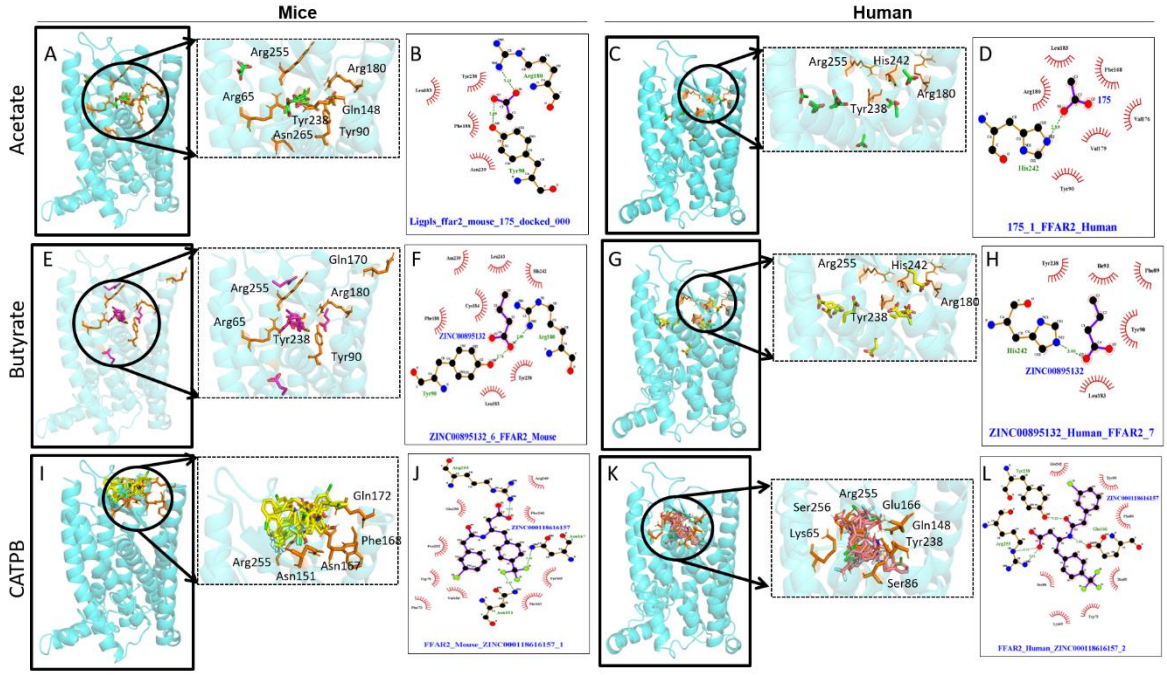


Fig. 4. Structural analyses of FFAR2 protein-ligand bindings with agonists – acetate (A-D) and butyrate (E-H) and an antagonist- CATPB (I-L). in the ribbon models (A, C, E, G, I, K) and two dimensional Ligplot images (B,D,F,H,J, K)) and of mice (A, B, E,F,I, J) and human (C,D,G,H,K,L).

[17,44], lipid metabolism [86], and adipogenesis [73,74] in maintaining body homeostasis. Detailed studies on binding of SCFAs with FFFAR2 of other species organisms are need to be done.

So, in both human and mice, SCFAs are associated with activation of FFAR2 in regulating biological function like incretin hormonal synthesis [23], metabolic syndrome [24,27,35] and occurrence of autoimmune diseases [19,137] in host. So, these findings provide opportunities to study in detail which biological functions are regulated by FFAR2 and simultaneously screening the synthetic molecules for effective activation of FFAR2 for effective biological response by either inhibiting the mutation or changing the structural form of the receptor.

DEFINITIONS:

Orthosteric ligands. The ligand which binds to a receptor at endogenous active site.

Allosteric ligands. The ligand which binds to a receptor other than endogenous site.

Allosteric agonist ligands. Allosteric ligand that activate the receptors in the absence of orthosteric ligands by binding other than on active site.

Ago-Allosteric ligands. The ligand binds allosterically to activate a receptor in the absence of an orthosteric ligand equals to an allosteric agonist and also activate the receptor in the presence of an orthosteric ligand as a positive allosteric modulator (potentiate agonist-mediated receptor response).

Inverse Agonist. An inverse agonist is a ligand that binds to the same receptor as an agonist but induces a pharmacological inhibitory response.

4.2. FFAR3

SAR data showed that human FFAR3 prefers saturated or ali-cyclic moieties of SCAs for ideal binding [128]. Histidine at 4-TM (His^{140}) and 6-TM (His^{242}) is important in deciding the binding efficacy of SCFAs in human FFAR3, as indicated by mutagenesis studies replacing these AAs with alanine [131,132]. SCFAs via FFAR3 regulate various biochemical, cellular and physiological function such as metastasis, hormone synthesis, gut motility, adipogenesis, lipolysis, apoptosis and others [138]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

[135,139,140]. Detail in-silco analysis on the binding efficiency of SCFAs with FFAR3 in mice, rodent or any other species need to be study.

Although the interactions of SCFAs with FFAR2/3 are similar, however, still shows a degree of selectiveness in these interactions [10,123]. In addition, these complex interactions can be resolved by designing alternative compounds that show higher efficacy and selectiveness for binding. [128] However, more comprehensive studies are required to define the biological functions of FFAR2/3 independent of compensative effects as well as there is critical need to develop specific compounds for activating FFAR2/3 with higher efficacy than SCFAs to exploit their therapeutic potential [123]. Following section describes few examples of synthetic compounds that bind with FFAR2/3.

5. Interaction of synthetic ligands with FFAR2/3

5.1. FFAR2

Synthetic ligands like CATPB and GLPG0974 act as allosteric antagonist to FFAR2 receptor by reducing Ca^{2+} and phosphorylated extracellular signal-regulated kinase (ERK)1/2 pathway [141-143]. For the first time CATPB interaction with human and mice FFAR2 are shown in **Figure 4**. Synthetic ligands like Compound 1 (Cmp1), ZINC03832747, compound 44, phenylacetamide 58 and Euroscreen compound series are orthosteric agonist of human FFAR2 [132,144,145]. Cmp1 is also an orthosteric agonist for mice FFAR2 [132,141]. Along with mutation at His^{242} site to alanine, mutation within the binding pockets at His^{140}, Val^{179}, Tyr^{90}, Tyr^{165}, and Tyr^{238} residual sites to alanine significantly reduced agonist property of Cmp1 for mice FFAR2 [146], as shown in **Figure 5.1**.

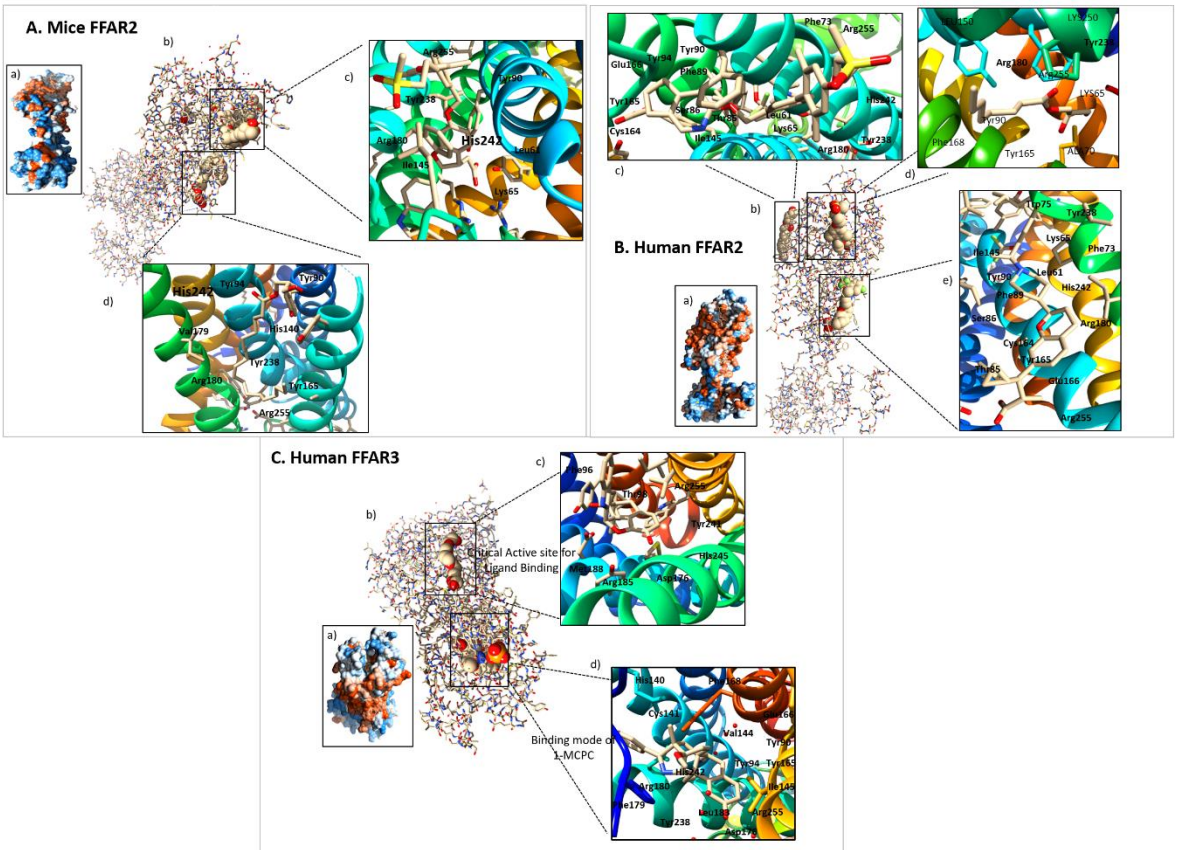


Figure 5. Mice (A) and Human (B,C) FFAR2 (A,B) and FFAR3 (C) Protein-Ligand interaction at orthosteric and Allosteric sites. **A) Mice FFAR2 Protein ligand Interaction** a) Hydrophobic model; b) Ball and stick model with c) Orthosteric binding site of C3 (Propionate), d) Allosteric binding site of Cmp1; **B) Human FFAR2 Protein ligand Interaction** a) Hydrophobic Model, b) Ball and stick model with c) Orthosteric binding site of 4-CMTB, d) Critical binding sites, e) Allosteric binding site of 4-CMTB; and **C) Human FFAR3 Protein ligand Interaction** a) Hydrophobic model, b) Ball and stick model, c) Critical Active sites for Ligand Binding, d) Binding mode of 1-MCPC.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

The CFMB (previously known as phenylacetamide 1) [147], AMG-7703 [148], and tiglic acid [136] allosteric agonists (that bind other than orthosteric site and activate receptor activity) [149] of human FFAR2. CFMB forms H-bond at Ile^{66}, Phe^{89}, Leu^{173}, Tyr^{238}, and Val^{259} residues [147]., while AMG-7703 forms H-bond at Ile^{66}, Phe^{89}, Leu^{173}, Val^{259}, Tyr^{90}, Ile^{145}, Asn^{239}, and His^{242} [148] with human FFAR2. Histidine residue at site (His^{242}) in human FFAR2 serves as a key residual site to classify whether a ligand will show allosteric or orthosteric activity [148]. 2CTAP, BTI-A-404 and BTI-A-292 are inverse agonist (a ligand bind to the receptor as an agonist but inhibit its pharmacological response) of human FFAR2 and reduced Ca^{2+} level via $G\alpha_q$ signaling [150,151]. But detailed information on structural and molecular interactions of 2CTAP, BTI-A-404, BTI- 292, and GLPG0974 with human FFAR2 are not available.

4-CMTB is an ago-allosteric modulator ligand for human FFAR2 as it increases the binding efficacy of SCFAs (like a positive allosteric modulators) and also activate the human FFAR2 receptor of its own (like a allosteric agonist) [148-150,152]. The ago-allosteric modulator, 4-CMTB binding interaction with human FFAR2 receptor is shown in **Figure 5.2**. The CFMB, phenylacetamide 2 and phenylacetamide 58 are allosteric agonist to mice FFAR2, but only demonstrated through biological phenomenon, however insilico studies remain unknown [128,145,147]. The chicken FFAR2 homology model has shown that four active residues are responsible for binding of vorapaxar ligand to FFAR2 receptor [91]. Three more AAs at Tyr^{246}, Met^{80} and His^{182} provide supports to this ligand-binding grooves [91].

5.2. FFAR3

A well-known human FFAR3 agonist, 1-MCPC forms H-bonds at different binding residues to activate FFAR3 receptor shown in **Figure 5.3** [125,136]. Pertussis Toxin (PTX) is a human FFAR3 inhibitor known to inhibit the FFAR3 receptors pharmacological and biological function via p38 and JNK (c-Jun N-terminal kinase) pathway [153]. Similarly, based on biological phenomenon, AR420626 and cyclopropanecarboxylic acid are selective allosteric agonist, and AR399519 and CF_3-MQC are antagonist for mice FFAR3, however detail *in-silico* analysis yet to be done [2,128,154]. To the best of our knowledge, so far no studies have directly addressed interactions of synthetic ligands with human nor rodent FFAR3, therefore this opens opportunities to study such in detail such interactions using dry and wet lab technologies.

Interestingly, at many instances, FFAR2 and FFAR3 activities are interchangeable and/or compensatory, this is because of similar chemical and structural characteristics. For example, substation of FFAR2 amino acid residues such as Glu^{166}; Leu^{183} and Cys^{184} with corresponding FFAR3 residues like Leu, Met and Ala using site directed mutagenesis favored the binding of FFAR3 agonists - 1-MCPC and 3-pentenoic acid, while these compounds were not able to bind wild type FFAR2 [128]. However, in human as well as in mice, the important source of SCFAs (known orthosteric ligands of FFAR2/3) is the host gut microbiota, which drive the next step to discuss the role of gut microbiota in SCFAs production in regulating the pharmacological and physiological function of host body through FFAR2/3 signaling.

4. Gut microbiome produces FFAR2/3 ligands- SCFAs

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

The gut microbiota is an important source of SCFAs that exhibit several health beneficial effects such as immune [34,43,54], metabolic [25,50,136,155,156] and neuronal [104,157] functions by activating FFAR2/3 signaling. The gut microbiota like *Bacteroides (B.) thetaiotaomicron, Akkermansia (A.) muciniphila, Bifidobacterium spp., Prevotella spp., Ruminococcus spp., Blautia hydrogenotrophica, Clostridium spp., Streptococcus spp.* produce acetate from pyruvate via acetyl-CoA and/or reductive acetyl-CoA pathway [157,158]. The propionate produced by *B. thetaiotaomicron, Roseburia spp., Firmicutes, Roseburia inulinivorans, Ruminococus spp., Clostridiales (C.) bactrium, Eubacterium (Eu.) spp., Coprococcus spp., Dialister succinatiphilus, Phascolarctobaterium succinatutens, A. muciniphila, Clostridium sp., Coproccus catus, Clostridium sp., Roseburia insulinivorans, Ruminococus spp., Eu. halli* from succinate, acrylate and/or propanediol pathways [157,158]. Similarly, butyrate is produced by *C. tyrobutyricum, Roseburia intestinalis, Eu. rectale, Roseburia insulinivorans, Clostridiales bacterium, Anaerostripes hadrus, Coprococcus spp, C. symbiosum, Faecalibacterium prasnitzii, Bacteroidetes spp., Coprococcus spp.* by butyrate kinase and/or butyryl-CoA:acetate CoA-transferase biosynthesis route [157,158]. The SCFAs produced by gut microbiota in intestine not only act on local intestinal cells like intestinal enteroendocrine cells [2], but also get absorbed from gut and circulate through portal and systemic blood to act on cells like monocytes [21], white adipocytes [14], neurons [16,23], cardiac cells [22], hepatocytes [16,17], skeletal muscle [18,19], alveolar cells [5,20,21,159], pancreatic cells [15], bone marrow [9] and splenocytes [20,21]. Chief biological functions of SCFAs activation of FFAR2/3 signaling, as these receptors are widely expressed in such cell types (**Figure 6)**. Indeed, SCFAs ameliorate obesity [25,31,50,140], diabetes [26,28,160], and colitis [33,43,117] which involves activation of activating FFAR2/3, indicating that gut microbiota derived SCFAs mediated activation of FFAR2/3 signaling plays a crucial role not only in maintain normal physiological and cellular functions but also protects from diseases.

5. Biological functions regulated by FFAR2/3 signaling

The biological functions regulated by FFAR2/3 signaling are immunity [34,54,115,161], gut hormonal synthesis [10,23,151], gut integrity [77] and neuronal function [2,102] to maintain the body homeostasis [2,10,23,134,138] (**Figure 6)** and described below.

5.1. FFAR2/3 in immune regulation

5.1.1. FFAR2

In human, FFAR2 exhibit anti-inflammatory response against metabolic diseases [21,54,162]. FFAR2 agonist (CFMB) treatment reduced pro-inflammatory response in to human monocyte by increasing phosphorylation of p38-mitogen-activated protein kinase (MAPK) signaling [21]. Moreover, FFAR2 knock-out (KO) mice show more severe inflammation in colitis, arthritis and airway inflammatory (asthma) mice, which indicate that FFAR2 signaling helps in reducing the proinflammatory response [34,39,163]. FFAR2 KO mice show enhanced neutrophil migration and proinflammatory cytokine secretion in the intestine [44,117,164]. Moreover, activation of FFAR2 by SCFAs ameliorates colitis in chronic dextran sodium sulphate (DSS)-induced colitis mice model, [68] (Table 2).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

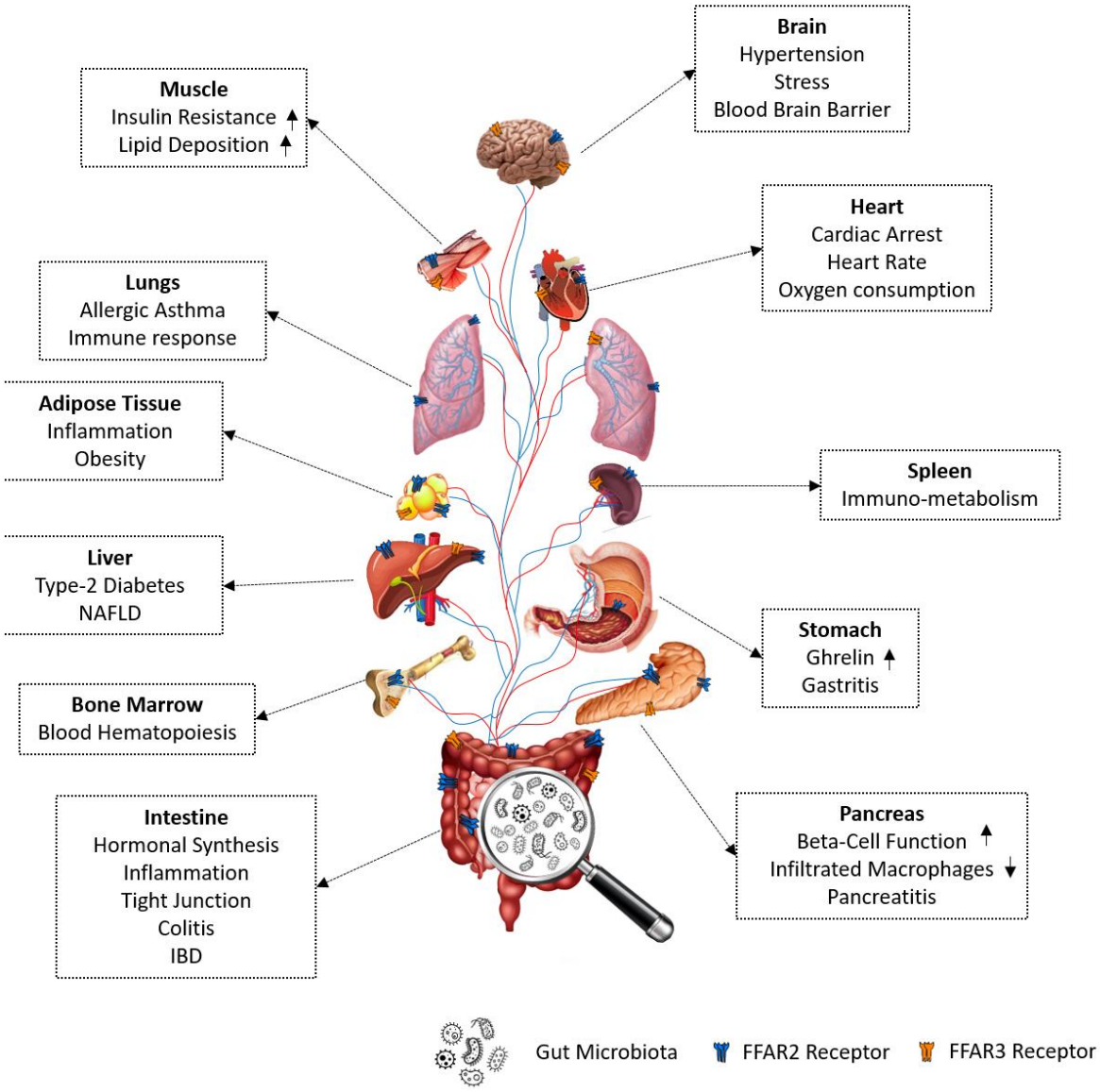


Figure 6. Biological Function of SCFA Receptors at different body parts

FFAR2 signaling activation lead to increase immunoglobulin (Ig)A (first line of defense against pathogens at the mucosal surfaces) production to protect intestinal epithelium against foreign pathogenic microbes invasion [116,165]. Sun et al. [160] showed that activation of FFAR2 signaling increases cathelicidin related antimicrobial peptide (CRAMP) production from pancreatic endocrine cells as protection against type-1 diabetes (T1D) [160]. In addition, butyrate mediated activation of FFAR2 signaling in mice chondrocyte exhibit anti-inflammatory activity by inhibiting the phosphorylation of NFκB (nuclear factor kappa-light-chain-enhancer of activated B cells), MAPK, AMPK-α (5' adenosine monophosphate-activated protein kinase) and PI3K (Phosphatidylinositol 3-kinase)/Akt (Protein Kinase B) pathway [166]. Moreover, FFAR2 activation by SCFAs reduce IECs graft-versus-host disease by activating nucleotide-binding oligomerization domain-like receptor protein 3 inflammasome (associated with IECs repairing by IL-18 secretion and maintaining integrity) [167]. FFAR2 also induces neutrophil chemotaxis through activation of P13Kγ, Rac2 (Rho family GTPase), p38-MAPK, and extracellular signal-regulated kinases (ERK) signal transduction pathway

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

[168]. On contradictory, FFAR2 KO mice with chronic DSS-induced colitis phenotype shows decrease in invasion of PMNs and cytokine keratinocyte chemoattractant synthesis as compared to wild type (WT) mice [41,42,44]. Also, FFAR2 KO mice reveal decrease in adaptive inflammatory response as compare to WT littermate in gout pathology [38]. This debatable immune modulation by FFAR2 signaling needs further studies, to understand its precise mechanism(s) and their importance in different disease pathologies.

5.1.2. FFAR3.

FFAR3 signaling activated by acetate and propionate reduces production of proinflammatory cytokine (Tumor Necrosis Factor alpha [TNF-α]) secretion [41], and enhances anti-inflammatory chemokines (C-X-C motif ligand 1 (CXCL-1) and CXCL-2) via enhancing the extra-cellular ERK1/2, p38-MAPK, PI3K or mTOR (The mammalian target of rapamycin) signaling [43,169,170]. In addition, FFAR3 expression increases on soluble fiber administration with decrease in macrophages, eosinophils, neutrophils migration and exhaled nitric oxide synthesis (eNOS) against asthma [39], so FFAR3 signaling enhances adaptive immune response. Moreover, in influenza infected mice, FFAR3 pathway increases anti-viral immunity activity on dietary fermentable fibers and SCFAs administration [115]. In addition, FFAR3 pathway stimulated by propionate reduces lungs allergic inflammation and total amount of IgE (antibody associated with allergic reaction) concentration in the serum [5]. Moreover, FFAR3 KO mice show lower immune response against *Citrobacter (C.) rodentium* infection with delayed in expression of interferon gamma (INFγ) (critical cytokine for innate and adaptive immunity against infection) through Raf (rapidly accelerated fibrosarcoma) which activates the MAPK/ERK pathway [43]. However, single-cell RNAseq of eosinophilic esophagitis patient T-cell exhibit higher expression of FFAR3 with increase Th2 cytokines (that exacerbate allergies) production [40,171]. These observations indicate that FFAR3 signaling involve in differential immune response of allergic reactions.

In mice macrophages (Raw 264.7), activation of FFAR3 signaling by SCFAs reduces the proinflammatory cytokines and increases nitric oxide synthase (iNOS) secretion [49]. Moreover, in human umbilical vein endothelial cells (HUVEC), FFAR3 mediated signaling reduces the LPS or TNFα stimulated atherosclerosis by inhibiting the proinflammatory cytokines and vascular cell adhesion molecule-1 synthesis on propionate and butyrate treatment [60,172]. However, another study reported that butyrate treatment in femoral bone marrow derived macrophages develops anti-microbial effect through histone deacetylases inhibitor (HDACi) pathway independent of FFAR3 [173]. So, epigenetic or FFAR2 immune response compensate FFAR3 immune signaling. However, in ruminant (*Capra hircus*) fed with high concentrate diet (60%) increases LPS and SCFAs production that activate FFAR2/3 signaling to produce cytokines and chemokines which in turn lead to cecal inflammation [174]. These results indicated that the role of FFAR3 signaling in regulating inflammation is controversial, and it may disease/ context dependent, hence further studies are needed to comprehend the role of FFAR3 signaling in immune modulation in disease specific manner.

Overall, these observations indicate that both FFAR2/3 are closely associated with complex mechanism of immune response [41,68,168,175-177], and cell specific responses in different diseases remain to be elucidated.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

5.2. FFAR2/3 in gut hormonal synthesis

FFAR2/3 signaling significantly contribute in gut hormone homeostasis through gut-hepatic [4,178] and gut-brain [2,157,179,180] axis regulate metabolic functions (**Figure 7)**. Incretin hormones like PYY and glucagon-like peptide 1 (GLP-1) secreted from L-cells (ileum and colon) have anorexigenic effect (reducing food intake) through enhancing expression of pro-opiomelanocortin (POMC) whereas suppressing agouti-related peptide (AgRP) and neuropeptide Y (NPY) in the hypothalamus of brain. While, ghrelin (secreted from X/A like cells in stomach) act as an orexigenic effect (increasing food intake) *via* increasing NPY/AgRP signaling [23]. Overall, FFAR2/3 play a vital role in maintaining homeostasis of neuropeptides (GLP-1, PYY, CCK, ghrelin) and neurotransmitters (catecholamine, serotonin and GABA) synthesis, and nutrient absorption [23,140,178], and detailed evidences are described below.

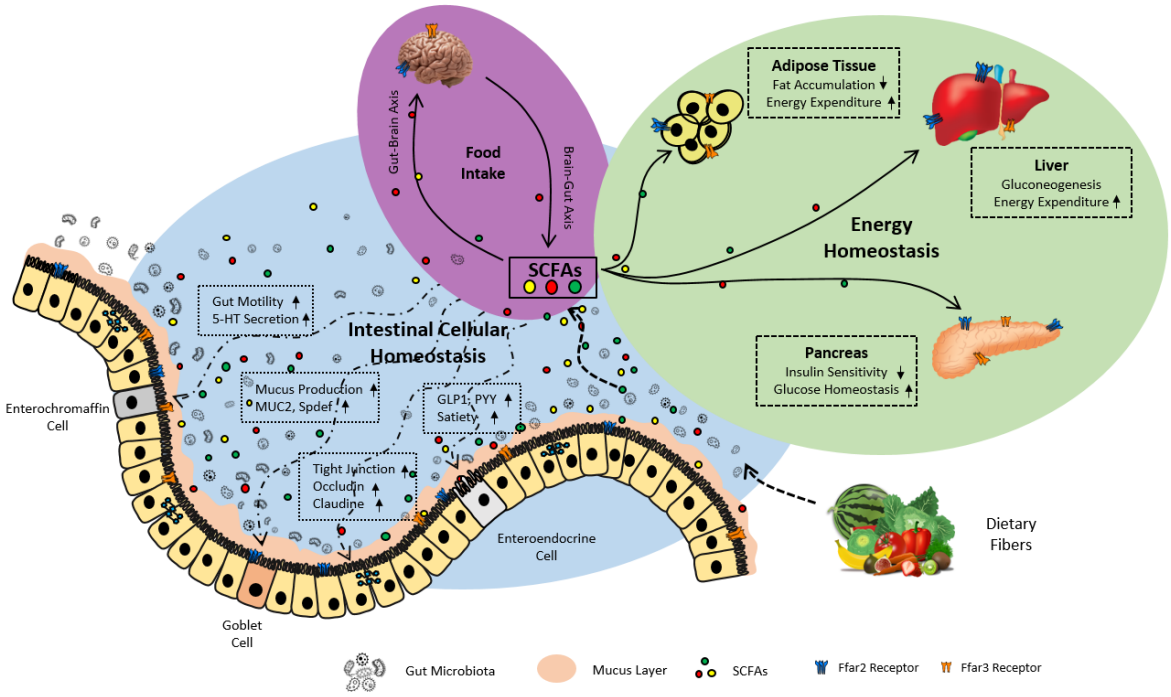


Figure 7. Importance of FFAR2/3 on Intestinal Cellular homeostasis in regulation of whole body energy balance

5.2.1. FFAR2

FFAR2 activation increases GLP-1 and PYY synthesis in human, rodents and guinea pig L-cells [61,75,181,182]. Tolhurst *et al*., [10] for the first time reported that activated FFAR2 signaling increases GLP-1 hormonal synthesis from L-cells of mice with increase in Ca^{2+} levels. In addition, FFAR2 KO mice show decrease in GLP-1 and insulin secretion leads to impair glucose tolerance even under SCFA treatment [10]. However, inulin (a prebiotics that promotes SCFAs production) feeding increases L-cells population in HFD-fed mice and protects obesity/T2D, while such effects of inulin were disappeared in FFAR2 KO mice [23], suggesting that FFAR2 is required for acetate action to prevent HFD-induced obesity/T2D. The activated FFAR2 controls blood glucose by increasing PYY

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

and GLP-1 [10]. In addition, FFAR2 agonist (CFMB) treatment to mice intestinal organoid directs more PYY and GLP-1 secretion with reduce cyclic adenosine monophosphate (cAMP) levels [23,183]. The novel FFAR2 antagonist like CATPB, BTI-A-404 and BTI-A-292 decreases the GLP-1 hormonal synthesis from NCI-H716 cells through downregulation of ERK, p38 MAPK, and NF-κB pathways [151]. These results profoundly indicate that FFAR2 signaling regulates GLP-1 and PYY secretion, and may pave the ways to consider FFAR2 as therapeutic target against diabetes, because GLP-1 increase is beneficial in regulation of blood glucose levels. However, in a rat study, FFAR2 agonist CFMB has no effect on colonic GLP-1 hormonal synthesis [184], indicating that either CFMB is not agonist for rat FFAR2 and or it plays differential role in rat intestine compared to human and mice.

In addition, FFAR2 signaling also involve in the GI tract buffering, especially on the conjunction of stomach and duodenum where acid of stomach poured down in the duodenum. A study showing that FFAR2 agonist phenylacetamide 1 increases the duodenal HCO_3^- secretion via activating 5-HT_4 receptor, and muscarinic M_1 and M_3 receptors [185], therefore balances the acidity coming from stomach in the duodenum.

5.2.2. FFAR3

Tolhurst *et al*., [10] also reported that GLP-1 hormone synthesis is regulated by FFAR3 signaling mediated through SCFA. GLP-1 and PYY significantly reduced in FFAR3 KO mice as compare to their WT littermates [3]. However, FFAR3 agonist (AR420626) increases GLP-1 release in mice colonic crypts [2]. In addition, co-administration of maltose and miglitol (α-glucosidase inhibitor) to mice increases plasma SCFA and GIP levels via FFAR3 as such effect was note seen in FFAR3 KO mice [186]. But such effect of dietary fibers on GLP-1 levels was not seen in antibiotic treated, germ-free and FFAR3 KO mice [186]. Activation of FFAR3 signaling increases the GLP-1 [10], while AR420626 (FFAR3 agonist) and AR399519 (FFAR3 antagonist) treatment to rat and AR420626 (FFAR3 agonist) treatment to mice intestinal organoid shows no effect on the synthesis of PYY and GLP-1 hormonal synthesis [23,184]. The exact reason behind these discrepant results is not known. Moreover, FFAR3 agonist (AR420626) treatment reduces enteropathy (ulcer formation and gastrointestinal bleeding) symptoms induced by indomethacin in rats by increasing in duodenal HCO_3^- and GLP-2 hormonal synthesis whereas FFAR3 antagonist (CF_3-MQC) counteract the AR420626 effect on reducing the enteropathy condition [154], indicating that the mucosal protective effect of AR420626 was mediated by FFA3 activation.

Even, increased synthesis of PYY and GLP-1 hormone by Roux-en-Y gastric bypass (RYGB) surgery leads to overexpression of both FFAR2 and FFAR3 in the intestine [187]. This indicate that incretin hormonal synthesis is associate with FFAR2/3 receptor signaling in response to metabolic syndrome in either direction. So, either through genetic mutational study on FFAR2/3 or their interactive action with targeted agonist and antagonist would help in exploring exact mechanism of action of FFAR2/3 against various gut hormonal comorbidities like obesity and T2D.

5.3 FFAR2/3 in intestinal epithelial integrity and inflammation

Emerging evidence indicate that the FFAR2/3 signaling significantly contribute in nutrient absorption [61,75] and helps to maintain the intestinal epithelial integrity [7,23] (**Figure 7)**, as described below**.**

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

5.3.1. FFAR2

FFAR2 signaling contributes in (i) maintaining intestinal integrity which in turn reduces leaky gut, (ii) regulating the colonic motility through intestinal 5-hydroxytryptamine (5-HT) release [64,75] and gut dysbiosis [64,75]. FFAR2 activation increases the expression of tight junction proteins (tight junction protein 1 [Tjp1], Occludin [Ocln], Claudine [Cldn]1) and mucus secreting markers like mucin (Muc)1 and Muc2 to maintain intestinal integrity [188], thereby reducing pro-inflammatory markers (interleukin [IL]-1β and TNF-α). In contrast, a significant decrease in mucin production (Muc2, Muc3, Muc4, and -Muc5b) was observed in the intestine of FFAR2 KO mice further indicating that FFAR2 KO mice have compromised gut barrier functions that were associated with reduced antimicrobial peptide synthesis (Reg3α, Reg3β, and Reg3γ), suggesting higher risk of microbial translocation [163]. Even in chicken, the modulated intestinal microflora by galactooligosaccharides increases the intestinal innate immune response, barrier function along with FFAR2 gene expression, suggesting FFAR2 receptors are involved in maintaining intestinal immune homeostasis [189]. However, antibiotic treatment reduces FFAR2 expression and increases in colonic epithelial permeability and inflammatory cytokines (TNF-α and IL-10) [77] in mice, further suggesting the role of FFAR2 in maintaining intestinal homeostasis. Also, FFAR2 KO mice model with dextran sodium sulphate (DSS)-induced colitis exhibit decrease in colon length, increase morbidity, increase daily activity index (DAI), inflammatory mediator myeloperoxidase, and with decrease in innate immunity markers like Toll-like receptors (TLR2 and TLR4) compared to their control FFAR2 WT littermate [34,69,161,164,184,190]. While, FFAR2 agonist reduces body weight gain, DAI, fecal Lipocalin-2 level (biomarker of intestinal inflammation) and pro-inflammatory cytokines (IL-6) and keratinocytes chemoattractant cytokine secretion from colonic mucosa of DSS-induced colitis mice, suggesting that FFAR2 agonism protects against colitis [69].

Also, FFAR2 KO-NOD mice have higher rate of T1D development as compare to FFAR2 WT-NOD mice [175]. However, acetylated high-amylose maize starch administration to FFAR2WT-NOD mice shows protection against diabetes but such effect was no seen in FFAR2 KO-NOD mice [175]. Furthermore, butylated high-amylose maize starch administration to FFAR2 KO-NOD mice show protection against diabetes due to increase in the population of CD4+Foxp3+ Treg cells in the colon [175]. At molecular level, via epigenetic-histone modification butyrate convert the naive $Fox3^-$ T-cells to $Fox3^+$ Treg cells through overexpression of FoxP3 protein, IL-10 and Helios transcription factor to provide protection against T1D (or autoimmune activity) by increasing the number of autoreactive T cells and Treg cells [175]. In human intestinal PBMC, FFAR2 agonist butyrate reduces gut permeability and protection against LPS-induced pro-inflammatory (IL-1β and TNFα) production [7,191]. SCFAs reduces colonic inflammation by decreasing the secretion of proinflammatory cytokines (IL-6 and IL-12), and chemokines from the intestinal epithelial cells and/or through increasing IgA and IgG (B cells) production and interacting with DCs in TNBS (2, 4, 6-trinitrobenzene sulfonic-acid- an intestinal inflammatory agent) and *C. rodentium* infection induced intestinal inflammation in FFAR2 KO mice [176,192]. However, inulin (a dietary fiber) feeding which increases SCFAs (ligands of FFAR2) also increase the expression of tight junction proteins independent of FFAR2/3 [23]. Also, activated FFAR2 signaling by natural indigenous fruit black raspberries increases the host immune response in gut of human and colorectal cancer mice ($Apc^{Min/+}$)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

model [193,194]. However, contradictory findings reported by Hatanaka et al. [195] showing that the FFAR2 signaling promotes occurrence of GIT tumorigenesis. This controversial results of FFAR2 in intestinal integrity is might be due to (i) epigenetic changes induced by SCFAs and/or (ii) compensatory response by FFAR3 signaling. Further, precise mechanistic studies to develop full understanding about the role of FFAR2 signaling in intestinal integrity are warranted.

5.3.2. FFAR3

FFAR3 maintains intestinal integrity by activating the cytokines and chemokines through MEK-ERK pathway [43]. In FFAR3$^{-/-}$ mice, inflammatory response was significantly reduces as compare to their WT [176]. Grape seed proanthocyanidins reduces the diarrhea occurrence by improving intestinal integrity and by shifting towards SCFAs-producing microbes (*Lactobacillaceae and clostridium*) in young swine model [196]. SCFAs also decreases intestinal permeability by increasing Ocln and FFAR3 mRNA expression in swine intestine [196]. The SCFAs treatment shows potential inhibitor action against LPS-induced pro-inflammatory (IL-1β, IL-6 and TNFα) production by activatingFFAR3, tested in FFAR3 KO mice signaling [7,176,191]. However, FFAR3 KO mice on treatment with TNBS shows reduce in immune response along with suppression of neutrophil infiltration [176], so apart from FFAR3 signaling intestinal inflammatory action is regulated by some other mechanism.

5.4. FFAR2/3 in neurophysiology

After the deorphanization, many research groups have reported that neither FFAR2 nor FFAR3 are expressed in CNS [8,102]. But recently it has been reported that low expression of FFAR2 is detected in the CNS which is limited to glia and neurons of the caudate, but FFAR2 can also be detected in cortical neurons and pituitary gland [197]. FFAR3 is expressed in peripheral nervous system (PNS), particularly sympathetic neurons of the superior cervical ganglion as a vasoconstriction phenotypic effect [22,102,103].

5.4.1. FFAR2

FFAR2 regulates the blood brain barrier (BBB) permeability [157,179]. Butyrate mediated activation of FFAR2 signaling and colonization of single bacterial strain *Clostridium tyrobutyricum* (responsible for production of butyrate) and *Bacteroides thetaiotaomicron* (mainly produce acetate and propionate) in germ-free mice decreases BBB permeability through boosting up Ocln mRNA expression in the frontal cortex and hippocampus [157]. Even FFAR2 KO mice shows severe microglia abnormality with increased dendritic lengths, number of segments, branching points, terminal points and cell volumes as compare to control mice suggesting that FFAR2 regulates microglial maturation and function [8]. However, multiple sclerosis (autoimmune neuro-inflammatory disease associated with CNS) patient and experimental autoimmune encephalitis (EAE) mice model induced by immunization of myelin oligodendrocyte glycoprotein show lower SCFA concentration and high expression of proinflammatory marker along with FFAR2 and 3 expression [198]. Further supported by clinical and histological score that the FFAR2/3 KO mice are more resistant to experimental autoimmune encephalitis (EAE) pathogenesis as compare to WT mice [198]. However, administration of SCFAs to EAE mice shows anti-inflammatory effect by increasing the IL-10+ T-cells and IL-10

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

expression in CNS tissues to suppress the inflammation. Thus, despite SCFAs beneficial effects on the CNS function, the mechanisms of SCFAs and FFFAR2/3 signaling to protect autoimmune CNS inflammation are not known [198]. As, SCFAs also function through inhibition of histone deacetylase and modulating cellular energy flux such as mitochondrial functions, may be responsible for such effects. However, these pathways are not yet established in EAE pathogenesis.

5.4.2. FFAR3

FFAR3 controls sympathetic neurons which in turn regulates whole body metabolic homeostasis [22]. FFAR3 is expressed in portal neurons and regulates propionate-induced gluconeogenesis via gut-brain axis [156]. In FFAR3 KO mice, catecholamine producing enzyme, tyrosine hydroxylase (TH) level is significantly lower that affects the neuronal growth [22]. The heart rate is reduced in FFAR3 KO mice which is associated with decreased norepinephrine release from sympathetic neurons, indicating that FFAR3 signaling regulates sympathetic neuronal functioning [22]. FFAR3 signaling activates $G_{\beta\gamma}$-phospholipase C (PLC)-β3-ERK1/2-synapsin 2-β at serine 426 pathway to enhance norepinephrine release from sympathetic nerve endings [104]. FFAR3-dependent synthesis of norepinephrine releases from synaptic vesicles helps to modulate energy expenditure of the host body [104]. Further, the treatment of mice with FFAR3 agonist- propionate, elevates the heart rate and oxygen consumption by increasing β-adrenergic receptor in ganglions [22]. In addition, FFAR3-signaling inhibit N-type calcium channels in neurons [103,199].

Won et al., [105] FFAR3 signaling activates $G_{\beta\gamma}$ signaling pathway and inhibits N-type Ca^{2+} channels, which in turn reduces neuronal catecholamine release in rat sympathetic nervous system. Moreover, in proximal colonic mucosa of rat, FFAR3 is associated with cholinergic-mediated secretory response in enteric nervous system [200]. Thus, FFAR3 is a potential target for treating neurogenic diarrheal disorder by reducing the nicotinic acetylcholine receptor (nAChR) activity [201]. Moreover, on treatment with FFAR3 synthetic agonist AR420626 suppresses nAChR or serotonin mediated motility changes with a consistent effect on FFAR3-stimulated anti-secretory effect [201]. FFAR3 expressing neurons in sub-mucosal and myenteric ganglionic plexus of small intestine regulates gut hormonal synthesis [2]. Mostly, in the distal part of small intestine (ileum), the FFAR3-expressing neurons reported to be express in substance P and somatostatin enteroendocrine cells derived from the CCK-secretin-GIP-GLP1-PYY-neurotensin lineage [2,180]. These evidence shows FFAR3 signaling just like FFAR2 is a promising therapeutic target for treating gut related disorders such as obesity, T2D, colitis and diarrhea, by honing gut-hormonal synthesis and balancing microbiome-gut-brain axis (**Table 3)**.

5.5. FFAR2/3 in adipogenesis and lipolysis

Several cellular and molecular pathways involved in adipogenesis, lipolysis, glucose homeostasis, insulin sensitivity and energy metabolism are regulated by FFAR2/3 signaling (**Figure 7)** [2,10,27,29,74,140,155]. FFAR2/3 signaling prominently modulates leptin secretion from adipose tissue to impact adipogenesis and dysglycemic conditions [138,155].

5.5.1. FFAR2

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

FFAR2 is responsible for energy accumulation in adipose tissues, adipogenesis and metabolic syndrome disease pathogenesis [114]. In-vitro (differentiated 3T3-L1 cells) and *in-vivo* (C57BL/6J mice) study on adipocyte has shown that FFAR2 increases adipogenesis [74]. In mice, acetate and propionate administration boost up FFAR2 expression in adipose tissues with reduce plasma FFAs level and decrease lipolysis [30,74,145]. Moreover, when FFAR2 KO mice fed with high fat diet (HFD) shows higher energy expenditure, plasma FFA level and higher food intake that leads to obesity as compare to WT mice [29,32,119]. However, activated FFAR2 signaling by SCFAs administration to diet induced obese (DIO) mice demonstrate reduce body weight by promoting beige adipogenesis and mitochondrial biogenesis with reduction of *Firmicutes*: *Bacteroidetes* ratio along with lower plasma FFA level [18,202]. Moreover, SCFAs treatment to adipose-specific FFAR2 KO transgenic (aP2-Gpr43TG) mice induces pro-inflammatory cytokine (TNF-α) in anti-inflammatory M2-type macrophages within adipose tissue milieu [203]. Apart from SCFAs, ketogenic metabolites acetoacetate activates FFAR2 activates ERK1/2 signaling in ketogenic condition (fasting or diabetic) to regulate energy homeostasis and maintains lipid metabolism [204]. During lactation, bovine adipocytes exhibit higher FFAR2 expression, indicates genetic switch-on of FFAR2 enhances adipogenesis to compensate high energy requirement of the animal during lactation [83].

Also, in the differentiated 3T3-L1 cells, FFAR2 activation by propionate enhances adipogenesis via peroxisome proliferator-activated receptor gamma (PPAR-γ2) pathway and the presence of FFAR2 siRNA (small interfering ribonucleic acid) inhibits the adipogenesis process [74]. In addition, in 3T3-L1 cells, FFAR2 allosteric agonist (phenylacetamide 1 and 2) suppresses the adipocyte lipogenesis indicating activated FFAR2 receptors reduces lipogenesis [145,147]. Moreover, in immortalized brown adipocyte cell line (IM-BAT), Rosiglitazone (anti-diabetic adipogenic drug) increases the FFAR2 expression via PPARγ-dependent manner to regulate adipogenesis [205]. In contrast, FFAR2 KO mice fed with HFD show lower body fat mass, improved glucose control, lower plasma lipids, increased body temperature with BAT density and lower WAT inflammation – indicating that FFAR2 deletion protects HFD-induced obesity/T2D [206]. Other studies show that acetate and propionate has no effect on adipogenesis in 3T3-L1 cells or mouse models and also no effect on either FFAR2 or FFAR3 expression [114,138]. A human study also reveals that FFAR2 expression in adipose tissues has no correlation with adipogenesis [207]. These observations indicates that the role of FFAR2/3 in adipose biology remain controversial and need further investigations.

5.5.2. FFAR3

Human multipotent adipose tissue-derived stem (hMADS) model reveals that activated FFAR3 by acetate significantly reduces lipolysis through decreasing hormone-sensitive lipase phosphorylation [14]. In mice, FFAR3 stimulated by gut microbiota derived SCFAs increases leptin production, hepatic lipogenesis and adipocyte adipogenesis [3]. Under HFD administration, FFAR3 KO male shows high body fat mass, plasma leptin level and blood glucose level as compare to female littermates [208]. In porcine, stimulated FFAR3 by butyrate administration enhances lipid accumulation and adipogenesis by upregulating glucose uptake and *de novo* lipogenesis through activation of Akt and AMPK pathways [86]. Moreover, FFAR3 signaling reduces blood pressure of the mice by increasing renin (angiotensin secreted from kidney in controlling blood pressure, and maintaining body fluid and electrolytes level) production [19]. Furthermore, FFAR3 triggered by SCFAs regulates intestinal gluconeogenesis via cAMP-activated pathway [156] and satiety signaling

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

through gut-brain axis [16,23], thereby controls whole body energy metabolism. Moreover, butyrate effects to regulate lipolysis depends on FFAR3, as PTX (known FFAR3 antagonist) treated 3T3-L1 (adipocytes) and Raw 264.7 (macrophages) show no effects on lipolysis, while butyrate alone increases lipolysis in these cells [49]. The leptin synthesis and FFAR2 expression found low in adipose tissues of FFAR3 KO mice [73], however, reason for these changes are not known. Overall, these findings indicates that FFAR3 plays a significant, but controversial role in regulating energy metabolism, however, precise mechanism(s) of remain elusive and needs further investigations.

5.6. FFAR2/3 in regulating pancreatic beta-cells proliferation and functions

Pancreatic beta-cells are crucial to regulate blood glucose homeostasis, by producing insulin. Therefore, maintaining and preserving beta-cell mass and functions remain critical. Beta-cell proliferation and differentiation is important for maintaining beta-cell, while beta-cell functions are important for efficiently releasing insulin in response to glucose. In T2D, beta-cell proliferation, differentiation and functions are deteriorated, that ultimately cause decrease insulin secretion and hyperglycemia in long-term diabetics. Coupling effect of FFAR2/3 receptors plays a fundamental role in regulation of glucose-stimulated insulin secretion (GSIS) [27,38,71,72,209] and directly or indirectly responsible for β-cell functions in regulating pathology T2D [18,32,210].

5.6.1. FFAR2

Starting from early embryonic stage, maternal gut microbiota-SCFA-FFAR2 signaling play a crucial role in regulating metabolic syndrome, as FFAR2 KO mice embryos have lower insulin and higher glucose level, and are more susceptible to obesity and diabetes in adulthood [211]. Also, FFAR2 KO mice on normal chow (NC) shows reduced β-cell mass and develops obesity and T2D characterized with increased glucose intolerance and FFA levels [29,31]. Also, activation of mice pancreatic β-cells- MIN6 by FFAR2 agonist (phenylacetamide 58) promotes proliferation and differentiation of β-cells and enhances insulin secretion [31,145]. In contrast, deletion of FFAR2 in Min6 and EndoC-βH1 cells (human pancreatic cell line) using siRNA, increases the insulin synthesis [27]. Thus, the role of FFAR2 in regulating beta-cell proliferation, differentiation and their functions remains elusive and further comprehensive studies are need to elaborate our understandings in this context.

5.6.2. FFAR3

Gut microbiota changes in obese humans are associated increased FFAR3/ Gi signaling to inhbit insulin synthesis [52]. These changes are further associated with epigenetic changes in FFAR3 receptor promotors (CpGs) and propensity of obesity and T2D, while lower methylation of FFAR3 promoter is associated with higher body mass index [26]. However, FFAR3 activation by butyrate increases human β-cells mitochondrial respiration, thus may be important to ameliorate beta-cell dysfunctions in T2D [212]. Also, in rodent, propionate stimulated FFAR3 signaling decreases the glucose oxidation and ATP/ADP ratio via $G\alpha_{i/o}$ pathway [50,213]. Opposite finding have been reported that either globally or pancreatic β-cell specific FFAR3 KO mice shows greater insulin secretion and improvement of glucose tolerance [27]. Similar type of results reported in Min6 and EndoC-βH1 cell lines where FFAR3 antagonist (PTX) treatment increases insulin secretion [27].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

Overall, these findings indicate that FFAR2/3 signaling is critical to regulate pancreatic beta-cells either by changing their proliferation, differentiation, insulin synthesis and regulating their functions in terms of GSIS, which in turn maintains better glucose homeostasis, however, the their precise role in regulating proliferation and differentiation are poorly understood.

7. Conclusion and Future directions

Dysbiotic gut microbiota with reduced SCFAs are related with suppression of FFAR2/3 signaling- that are known to regulate an array of biological pathways participation energy metabolism, adipogenesis, appetite control, intestinal cellular homeostasis, gut motility, glucose metabolism and inflammatory response. Alterations in these biological pathways are hallmarks of several human diseases such diabetes, obesity, IBS/IBD, crohn`s disease, atherosclerosis, gout, asthma, cardiovascular diseases, arthritis, hypertension and colitis, therefore, targeting FFAR2/3 signaling can provide promising therapeutic strategies for these human diseases. The immune cell during metabolic diseases like obesity and T2D causes chronic inflammatory response which provides an insight crucial mechanism for further disease progression. However, the role of FFAR2/3 signaling in these diseases remain controversial and need to further studied for better understanding their role to devise the therapeutic importance of FFAR2/3 agonist/antagonists. For example, majority of studies showing that activation of FFAR2/3 signaling ameliorates obesity/T2D pathology, however, some studies shows the opposite. Such as, HFD feeding to FFAR2 KO mice shows improved oral glucose tolerance test (OGTT) and insulin sensitivity along with lower fat mass and increased lean mass compared to wild type (WT) mice [27,29,32], indicating that the deletion of FFAR2 protects HFD-induced obesity/T2D. In agreement to this, the mRNA and protein expression of FFAR2 has no correlation with insulin secretion in T2D patients [52,214]. Therefore further studies are critically need to develop better understanding about the role of FFAR2/3 in regulating metabolic functions, and pathology of obesity/T2D.

The pharmacological modification of these SCFAs-receptors by endogenous or synthetic ligands provides an opportunity to counteract these gastrointestinal disorder in humans. However, overlapping expression of FFAR2 and FFAR3 in the same tissues/cells, and their similar affinity to specific endogenous ligands develops puzzled outcomes to understand the role of FFAR2/3 in particular biological functioning. Thus, the future studies must aim to develop highly specific and efficacious small molecules to modulates pharmacological actions of FFAR2/3 signaling, and and can display a promising strategy to prevent, manage and/or treat human diseases like diabetes, obesity, crohn`s disease, atherosclerosis, gout, asthma, cardiovascular diseases, arthritis, hypertension and colitis.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

Table 1. Expression of FFAR2 and FFAR3 in different species cell lines

S N	Cell line/Type	Species	Tissues/Cells	Expression		Reference
				FFAR2	FFAR3	
1	GLUTag	Mice	Intestinal Enteroendocrine Cells	Yes	Yes	Author*,[10,140]
2	Neuro2A	Mice	Brain	Barely	Barely	[22]
3	Cardiomyocytes	Mice	Heart	Yes	Yes	[22]
4	INS1	Rat	Pancreatic Islet	Yes	NA	[24,31]
5	CMT93	Mice	Rectal Cell	Yes	Yes	Author*
6	MEFs	Mice	Embryonic Fibroblast	Yes	No	[29]
7	THP-1	Human	Monocyte	Yes	Yes	Author*
8	3T3-L1	Mice	Adipose Tissue	Yes	Yes	Author*,[29,30,51,74,138]
9	Min6	Mice	Pancreatic Endocrinal Cells	Yes	Yes	[15,31,72,73,119,212]
10	HCT8	Human	Colon	No	NA	[33]
11	HCT116	Human	Colon	No	Yes	[33,100]
12	HT-29	Human	Colon	Yes	NA	Author*,[7,33]
13	SW480	Human	Colon	No	NA	[33]
14	Hepa1-6	Mice	Liver	Yes	Yes	Author*
15	SW620	Human	Colon	No	NA	[33]
16	CBS	Human	Colorectal	No	NA	[33]
17	FET	Human	Colon	No	NA	[33]
18	H9C2	Rat	Heart/Myocardium	Yes	Yes	Author*
19	MOSER	Human	Colon	No	NA	[33]
20	Caco-2	Human	Colon	Low level	NA	Author*,[7,33]
21	Raw264.7	Mice	Macrophages	Yes	NA	Author*,[28]
22	MCF7	Human	Mammary Gland	Yes	Yes	[28,63]
23	HEK293T	Human	Embryonic Kidney	Yes	Yes	[28,52,104,215]
24	AML-12	Mice	Liver	Yes	Yes	Author*
25	HeLa	Human	Cervix	Yes	NA	[28]
26	HTLA	Human	An HEK293 cell line stably expressing a tTA-dependent luciferase reporter	Yes	Yes	[215]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

			and a β-arrestin2-TEV fusion gene			
27	CHO-K1	Hamster	Ovary	Yes	Yes	[52,53,120,150]
28	WTA11	Hamster	A CHO-K1 cell line coexpressing mitochondrial apoaequorin and G16	Yes	Yes	[52]
29	COS-7	Monkey	Kidney	Yes	Yes	[52]
30	3T3-L442A	Mice	Adipose Tissue	Yes	Yes	[51]
31	HFF11	Human	HeLa expressing GPCR based on reported plasmid, pcFUS3	Yes	NA	[53]
32	βTC1	Mice	Pancreatic β-cells	Yes	Yes	[72]
33	βTCtet	Mice	Pancreatic Islet β-cells	Yes	Yes	[72]
34	SK-N-SH	Human	Brain	Yes	Yes	Author*
35	L-10	Mice	Lymphoid Cells	Yes	No	[72]
36	αTC1	Mice	Pancreatic α-cells	Lesser Extent	No	[72]
37	AR42J	Rat	Pancreatic Exocrine	Lesser Extent	No	[72]
38	NIH-3T3	Mice	Embryonic Cells	No	No	[72]
39	Ltk	Mice	Adipose fibroblast cell	Yes	No	[72]
40	Ob-Luc	Mice	Adipocytes	NA	Yes	[98]
41	NCI-H716	Human	Intestinal Endocrinal L-cell	Yes	Yes	[106,136]
42	CMEC/D3	Human	Brain Endothelium	NA	Yes	[62]
43	C2C12	Mice	Muscle	Yes	Yes	Author*
44	HBMEC	Human	Primary Brain Microvascular Endothelial Cells	NA	Yes	[62]
45	βTC3	Mice	Pancreatic β-cells	Yes	No	[25,119]
46	SW872	Human	Liposarcoma	Yes	No	[132]
47	hMADS	Human	Adipose tissue-derived stem cells	Yes	Yes	[14]
48	HuTu-80	Human	Duodenum Epithelium	Yes	Yes	[136]
49	HEPG2	Human	Liver	Yes	Yes	Authors*
50	BaF3	Mice	B Lymphocytes	Yes	Very low	[17]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

51	U937	Human	Myeloid Lymphocytes	Yes	NA	[17]
52	K562	Human	Bone Marrow	Very low	NA	[17]
53	C2BBe1	Human	Clone of Caco-2	Yes	NA	[7]
54	NCM-640	Human	Colon Epithelial cells	Very Low	NA	[7]
55	T-84	Human	Colon Epithelial cells	Very Low	NA	[7]
56	Jurkat T cells	Human	T-Lymphocyte Cells	NA	Yes	[40]
57	HRCEs	Human	Kidney tissues	Yes	Yes	[153]
58	HuH-7	Human	Hepatocellular	Yes	Yes	[139]
59	JHH-4	Human	Hepatic cell	Yes	Yes	[139]
60	HLE	Human	Liver cells	Yes	Yes	[139]
61	MDA-MB-231	Human	Breast Epithelial cells	Yes	Yes	[135]
62	MDA-MB-436	Human	Breast Epithelial cells	Yes	Yes	[135]
*Authors- we have confirmed the expression in our lab.						

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

Table 2. Physiological function of FFAR2 in Human and Mice

S. No.	Tissue/Organ	Research Findings	Ref.
Human			
1	Intestinal L-cells	-Secrete GLP-1 and PYY in response to glucose via FFAR2 signaling.	[2,10,75,216,217]
2	Primary Neutrophils	-Cmp1 and CATPB function as an agonist and antagonist for the neutrophil FFAR2 respectively. -Cmp1 and acetate activates the phospholipase C-inositol phosphate 3 (IP_3) Ca^{2+} signaling while CATPB inhibits it. -Cmp1 act as a potent activator of the NADPH-oxidase in TNF-α-primed neutrophils with increased release of superoxide. -Moreover, Cmp1 triggered NADPH oxidase activity was inhibited by PTX.	[218]
3	Primary Monocytes	-Non-responders of Cmp1 shows no transient rise in intracellular Ca^{+2}. -Human monocyte FFAR2 reduces inflammatory cytokine expression in response to acetate. -FFAR2 modulates p38-MAPK, Akt and ERK signaling in response to acetate and FFAR2 agonist (CFMB).	[21,169,218]
4	Primary Lymphocytes	-Non-responders to Cmp1 with no transient rise in intracellular Ca^{+2}.	[21,218]
5	Peripheral blood mononuclear cells (PBMCs)	-mRNA expression of FFAR2 upregulate in PBMCs in Type 1 Diabetes (T1D) patient via NFκB. -Overexpression induced cell apoptosis through ERK signaling. -Stimulated PBMCs for cytokine production in the presence of lipopolysaccharides (LPS) with and/or without acetate along with anti-FFAR2 antibody.	[28,41,218]
6	Primary Adipocytes	-FFAR2 expressed in the human multipotent adipose tissue-derived stem cells (hMADS). -SCFA acetate (luminal and systemic) are responsible for the antilipolytic response. -Treating with G_i-sensitive PTX inhibitors prevents anti-lipolytic response develop by acetate. -A mixture SCFA reduces plasma FFA in DIO mice along with beige adipogenesis marker. -So, colonic or systemic acetate modulation helps in improving the insulin resistance in human adipocytes via FFAR2 mediated attenuation of HSL phosphorylation.	[14,18,28,41]
7	Colon	-Luminal propionate (propionate) stimulates FFAR2 pathway through PYY mediation confirmed by Y1 and Y2 antagonist (BIBO3304 and BIIE0246).	[14,18,219]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

		-FFAR2 signaling expressed evenly in the entire intestine mostly at colon in the presence of FFAR2 agonist PA.	
Mouse/Rodent			
1	Pancreatic β-cells	-mRNA expression of FFAR2 upregulated through increase in pancreas β-cell expansion. -Increased β-cell contributes to more insulin secretion. -FFAR2 KO mice reduces gestational pancreatic β-cell expansion during pregnancy. -FFAR2 KO mice gestational glucose tolerance worsened even under antibiotic treatment and further deteriorated during second pregnancy. -Antibiotic modulation of gut microbiota does not disrupt the contribution of FFAR2 to gestational glucose tolerance. - FFAR2 act as a novel target for β-cells adaptation to pregnancy-induced insulin resistance during to maintain normal glucose homeostasis. -FFAR2 a novel therapeutic target to stimulate β-cell growth and Proliferation.	[24,96,220,221]
2	Primary Pancreatic Islet	--SCFAs like Acetate, propionate and butyrate administration has no effect on insulin and glucagon secretion regardless of glucose level. -CFMB (FFAR2 agonist) has significant effect in increasing the somatostatin and insulin secretion whereas no effect was observed in glucagon synthesis. -Mediate an inhibition of insulin secretion by coupling to Gi-type G Proteins -Under type-2 diabetic condition acetate concentration increases in pancreatic islet and systemic circulation -FFAR2 antagonist might increase insulin secretion in type-2 diabetes -Double knock-out of FFAR2 and FFAR3 altered the glucose tolerance in diabetic condition.	[27,222]
3	Ileum	-Bacterial metabolites, propionate, activate ileal mucosal FFAR2 to decrease hepatic glucose production. -Propionate stimulate GLP-1r dependent neuronal network to regulate glucose production activated through ileal FFAR2 signaling. -Regulate glucose homeostasis.	[27,178]
4	Macrophages	-Inducing apoptosis of infiltrated macrophages to pancrease through upregulation of FFAR2. -Improved glucose homeostasis in diabetic mice by treating with FFAR2 agonist, acetate and phenylacetamide 1.	[28,178]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

5	Peripheral blood mononuclear cells (PBMCs)	- Dextran sodium Sulphate (DSS) -induced colon shortening, mucosal thickness, inflammatory cell infiltration and crypt damage were ameliorated by acetate treatment in C57BL/6 mice. -Stimulated PBMCs in FFAR2 KO mice for cytokine production in the presence of lipopolysaccharides (LPS) with and/or without acetate. -DSS-induced colitis is exaberated in FFAR2 KO mice through increase in pro-inflammatory cytokines like TNF-α and IL-17 with decrease of anti-inflammatory cytokine IL-10 in the colonic mucusa.	[28,41]
6	Neutrophil	-FFAR2 recognizes propionate and butyrate, and expressed abundantly in polymorphonuclear (PMN) leukocytes. -FFAR2 mediated SCFA-induced chemotaxis through p38 MAPK signaling pathway. -Inhibiting FFAR2 mediated signaling a promising way for inhibiting the migration of PMN at the site of intestinal inflammation. -Under influenza infection, in FFAR2 KO along with wild type mice showed decrease neutrophil infiltration to airway.	[42,115]
7	Immune Cells	-FFAR2 KO mice develops unresolving or exaberated inflammation in colitis, arthritis and asthma mice model. -FFAR2 KO mice shows inflammatory action related to increase in the production of inflammatory mediators by increased in immune cell recruitment. -Germ-free mice, which are devoid of bacteria and express little or no SCFAs, showed similar dysregulation of certain inflammatory response. -SCFA-FFAR2 interaction has profound effect on normal resolution of certain inflammatory response with a molecular link between diet, gastrointestinal bacterial metabolism and immune response.	[34,39,42]
8	Monocytes	-Mice monocyte showed increased in IL-1α and IL-1β cytokine expression in response to acetate. -Even in FFAR2/3 KO mouse monocyte display elevate cytokine response on treatment with SCFAs. -SCFA does not act through FFAR2 to modulate mice monocyte inflammatory responses.	[21,34]
9	L-cells	-GLP-1 synthesis was enhanced in the presence of phosphodiesterase inhibitor isobutyl methyl xanthine (IBMX). -FFAR2 expression in small intestine and colonic L-cells as compare to non-L-cell population. -Induces GLP-1 and PYY secretion via glucose dependent mechanism. -SCFAs triggered Ca^{2+} elevation in L-cells with enhanced GLP-1 and PYY secretion through G_q-mediated pathway, implicating FFAR2 signaling involvement.	[10,21,217,223]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

		-Synthetic phenylacetamide agonist of FFAR2, CFMB, mobilizes more intracellular Ca^{2+} in L-cells and elevate GLP-1 hormone secretion, in the presence of DPPIVi but not in its absence in mice.	
10	Colonic Mucosa	-FFAR2 express in the colonic mucosa -Withdrawal of ceftriaxone antibiotic leads to reduction in SCFA concentration and increase in increased number of conditionally pathogenic *Enterobacteria*, *E. coli*, *Clostridium*, *Staphylococcus spp*. and hemolytic bacteria in colonic gut. -FFAR2 immune regulation mechanism get hamper with increase in cytokine concentration in colonic mucosa. -Increase histopathology condition of colitis with goblet cell dysfunction, colonic dilatation and wall thickening, ultimate leads to IBD.	[77]
11	Enterochromaffin cells	-FFAR2 agonist PA1 (Phenylacetamide 1) in a dose-dependent manner stimulate HCO_3^- secretion, even prior exposed to DPPIV inhibitor NVP728. -HCO_3^- secretion stimulate by activated FFAR2 through muscarinic and 5-HT_4 receptor signaling rather than through VIP, CCK and GLP-2 pathway. -Moreover, SCFAs (mostly acetate) activate FFAR2 and FFAR3 followed by 5-HT and GLP-2 release.	[185]
12	Mast cell	-Rat intestinal lamina propria mast cells expressed FFAR2 along with 5-hydroxytryptophan (5-HT). -The activated mucosal FFAR2 act on the nearby nerve endings at 5-HT_3 serotogenic receptors. -SCFAs stimulate PYY and 5-HT secretion from ileum and colonic endocrine cells by activating FFAR2 receptor.	[75,185]
13	Stomach	-The villi and microvilli of gastric brush cells reveal expression of FFAR2 (at gene and protein level) in the mice stomach.	[6,75,183]
14	Lungs	-Expressed in the mice lungs. -SCFAs modulate allergy airway inflammation in mice lungs via FFAR2 signaling.	[5,6,159,183]
15	Muscle	-Expressed FFAR2 in smooth muscle cells of small resistance vessels. -SCFAs produced from gut microbiota modulate the blood glucose level.	[5,19]

FFAR2: Free fatty acid receptor 2; GLP-1: Glucagon-like peptide 1; PYY: Peptide YY; Cmp1: Compound 1 (3-benzyl-4-(cyclopropyl-(4-(2,5-dichlorophenyl)thiazol-2-yl)amino)-4-oxobutanoic acid; ERK: Extracellular signal-regulated kinase); CATPB: (S)-3-(2-(3-chlorophenyl)acetamido)-4-(4-(trifluoromethyl)phenyl)butanoic acid; GLPG0974: 4-[[(2R)-1-(1-benzothiophene-3-carbonyl)-2-methylazetidine-2-carbonyl]-[(3-chlorophenyl)methyl]amino]butanoic acid; IP_3: Inositol phosphate 3; NADPH: Nicotinamide adenine dinucleotide phosphate; TNFα: Tumor Necrosis Factor alpha; PTX: Pertussis toxin; MAPK: Mitogen-activated protein kinase; Akt:

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

Protein Kinase B; ERK: Extracellular signal-regulated kinase; CFMB: [(S)-2-(4-chlorophenyl)-3,3-dimethyl- N-(5-phenylthiazol-2-yl)butamide; PBMCs: Peripheral blood mononuclear cells; T1D: Type-1 Diabetes; LPS: Lipopolysaccharides; hMADS: human multipotent adipose tissue-derived stem cells; SCFA: Short chain fatty acids; FFA: Free fatty acids; DIO: Diet induced obesity; HSL: Hormone-sensitive lipase; FFAR3: Free fatty acid receptor 3; GLP-1r: Glucagon-like peptide 1 receptor; DSS: Dextran sodium Sulphate; KO: Knock out; LPS: Lipopolysaccharides; IL: Immunoglobulin; PMN: Polymorphonuclear; SCFA: Short chain fatty acid; IBMX: Isobutyl methyl xanthine; DPPIVi: Dipeptidyl peptidase 4 inhibitor; PA1: Phenylacetamide 1; ACh: Acetylcholine; 5-HT: 5-Hydroxytryptophan; 5-HT_3: 5-Hydroxytryptophan type 3; HCO_3^-: Bicarbonate: VIP: Vasoactive intestinal peptide; CCK: Cholecystokinin; GIP: Glucose-dependent insulinotropic peptide; IGN: Intestinal Gluconeogenesis

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

Table 3. Physiological function of FFAR3 in Human and Mice/Rodent

S.No.	Tissue/Organ	Research Findings	Ref.
Human			
1	Intestinal L- cells	-Secrete GLP-1 and PYY in response to glucose.	[2,10,75,216]
2	Intestinal I- cells	-Secrete Cholecystokinin (CCK) in response to glucose.	[2]
3	Intestinal K- cells	-Secrete glucose-dependent insulinotropic peptide (GIP) in response to glucose.	[2]
4	Colon	-No effect of propionate response on Intestinal Gluconeogenesis (IGN) genes (G6PC, PCK1, MUT) expression with either FFAR2 agonist like tiglic acid (TA) or FFAR3 agonist i.e,1-methylcyclopropanecarboxylic acid (MA). -IGN gene expression increases by butyrate mediated through cAMP pathway but not via G_i- nor G_q pathway. -Neither Gi- nor Gq-sensitive inhibitors (PTX and U73122) able to reduce the IGN gene expression induced by butyrate.	[156]
5	Monocyte	-Human monocyte FFAR3 reduces cytokine expression in response to acetate. -The receptor modulates p38-MAPK signaling in response to acetate and FFAR3 agonist (AR420626).	[21]
6	Adipocytes	-FFAR3 expressed in the human multipotent adipose tissue-derived stem cells (hMADS). -Acetate is responsible for the antilipolytic response luminal and systemic level. -Rosiglitazone increases the expression of FFAR3. -FFAR3 stimulation develop anti-inflammatory action targeting TNFα and IL-1β. -Treating with G_i-sensitive PTX inhibitors prevents antilipolytic response develop by acetate. -Colonic or systemic acetate modulation helps in improving the insulin resistance in human adipocytes via FFAR3 mediated attenuation of hormone-sensitive lipase (HSL) phosphorylation.	[14,224]
7	Enteric Neurons	-FFAR3 agonist, AR420626 response at colon mucusa showed monophasic reductions in short-circuit currents (Isc) and sensitive to neurotoxin tetrodotoxin (TTX).	[2,219]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

		-At submucosal and myenteric neuronal plexus, the FFAR3 is colocalized with Vasoactive intestinal polypeptide (VIP). - FFAR3 antagonist AR399519 inhibits FFAR3 agonism activity in entire colonic region.	
Mouse/Rodent			
1	Pancreatic α- and β-cells	-FFAR3 is transcribed from the promoter of the GPR40. -The expression is mediated via an internal ribosomal entry site (IRES) located in the intergenic region of a bicistronic mRNA. -Helps in proper understanding in the identification of therapeutic target.	[15,27,72,209]
2	Primary Pancretic Islet	-FFAR3 expression in murine pancreatic islet -Leads to reduction of insulin secretion by coupling to Gi-type G Proteins in type-2 diabetic condition. -Locally to islet as well as in systemic circulation acetate concentration increases. -So, in type-2 diabetic condition FFAR3 antagonist may increase insulin secretion	[15,27,222]
3	Primary Pancreatic Islet	-Infusion of Acetate, propionate and butyrate has no profound effect on insulin and glucagon secretion regardless of glucose level. -Whereas, FFAR3 agonist Compound 4 (N-(2,5-dichlorophenyl)-4-(furan2-yl)-2-methyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide) has significant effect in increasing the somatostatin and insulin secretion but showed no effect on glucagon synthesis.	[222]
4	Sympathetic Nervous System (SNS)	-Expressed in the rodent SNS especially at Superior cervical ganglia (SCG) and Celiac-mesenteric Ganglia (CSMG). -Induced variable $I_{Ca^{2+}}$ modulation activity by sodium propionate in the $FFAR3^{+/+}$ mice. -Moreover, along with acetate and propionate, ketogenic metabolites β-hydroxybutyrate (BHB) produced voltage dependent reduction of N-type Ca^{2+} channel in SNS. -FFAR3-expressing neurons from reporter mice expressed decrease in $Ca_v2.2$-FFAR3 inhibitory coupling variability.	[102,103,105]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

		-FFAR3 is expressed primarily in neurons with a vasoconstrictor phenotype.	
5	Superior cervical ganglia (SCG)	-Propionate enhances the norepinephrine (NE) release from primary-cultured mice SCG. -Pretreatment with $G_{i/o}$ pathway sensitive-PTX; Gβγ inhibitor-Gallin; PLC inhibitor U73122 and MEK inhibitor U0126 significantly reduces NE secretion indicating the involvement of $G_{i/o}$, Gβγ, PLCβ3 pathway in hormonal secretory function. -Treatment with $G\alpha_{i/o}$ inhibitor NF023 shows no inhibition of NE release, so FFAR3 response is independent of $G\alpha_{i/o}$ pathway. -Further, treatment with siRNA against either PLCβ3 or ERK1/2 decreases the expression NE protein by more than 80%. -So, SCFA receptor FFAR3 is coupled with $G_{i/o}$ protein, to release NE via Gβγ-PLCβ3- ERK1/2-synapsin 2 pathway.	[104]
6	Intestine	-IGN induction is mediated by propionate through gut-brain axis. -Dietary propionate leads to c-Fos (neuronal activation marker) activation in the hypothalamic region which receives neuronal signal from both parabrachial nucleus (PBN) and dorsal vagal complex (DVC), mostly paraventricular nucleus (PVN), the lateral hypothalamus (LH) and the arcuate nucleus (ARC) of hypothalamus.	[156]
7	Intestinal Enteroendocrine Cells	-Acetate, propionate and butyrate administration in mice protect against diet-induced obesity and insulin resistance. -Propionate and butyrate but not acetate induce gut hormones and reduces food intake. -Butyrate had minor effect in stimulation of GLP-1 through FFAR3. -FFAR3 KO mice shows normal body weight and glucose homeostasis, indicating some additional mediators are involves in these mechanism. -FFAR3 KO mice shows impair GLP-1 synthesis with altered in mRNA expression of Glucagon, PYY and active GLP-1 peptide.	[10,140,155,208]
8	Monocytes	-Mice monocyte shows increase in IL-1α, IL-1β and GM-CSF cytokine expression in response to acetate.	[21]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

		-Even in FFAR2/3 KO mouse monocyte displays elevate cytokine response on treatment with SCFAs. -So, SCFA does not act through FFAR2 to modulate mice monocyte inflammatory responses.	
9	Neutrophil	-FFAR3 pathway is associated with airway neutrophil response subjected to influenza infection verified in FFAR3 KO mice.	[115]
10	Bone marrow	-FFAR3 KO mice produce less monocytes and interstitial macrophages from the bone marrow in response to butyrate	[115]
11	Ileum and Colon	-Moreover, dietary (Flaxseed) fibers restructured the gut microbiota with proliferation of the genera *Bifidobacterium* and *Akkermansia* reduces fat mass and show improve tolerance to intraperitoneal and oral glucose via FFAR3. -Microbiota is associate with increase SCFA production acting through FFAR3 signaling. -Through selective FFAR3-agonist, AR420626 showed greatest efficacy of FFAR3 at distal regions of intestine to protect mice from diet induced obesity by preventing a reduction in energy expenditure induced by an HFD.	[155,201,219]
12	Colonic Mucosa	-FFAR2 express in the colonic mucosa -Withdrawal of ceftriaxone antibiotic leads to reduction in SCFA concentration and increase number of conditionally pathogenic *Enterobacteria*, *E. coli*, *Clostridium*, *Staphylococcus spp*. and hemolytic bacteria in colonic gut. -FFAR2 immune regulation mechanism get hampered with increase in cytokine concentration in colonic mucosa. -Increased histopathology condition of colitis with goblet cell dysfunction, colonic dilatation and wall thickening, ultimate leads to IBD.	[77]
13	Duodenum L- cells	-FFAR3 is colocalized with GLP1 and expressed in L cells. -SCFAs (mostly acetate) activate FFAR2 and FFAR3 followed by 5-HT and GLP-2 release.	[185]
14	Enteric Neurons	-FFAR3 agonism (by AR420626) at descending colon mucusa was inhibited by neurogenic sensitive tetrodotoxin (TTX).	[2,200,219]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

		-FFAR3 agonist activity is sensitive to acetylcholinergic (ACh) neurotransmission in rat colon mucosa. -ACh muscarinic antagonist atropine, nicotinic sensitive hexamethonium, FFAR3 antagonist AR399519, GLP1 antagonist Ex(3-39) or calcitonin gene related peptide (CGRP) blocker BIBN4096 abolished FFAR3 agonism activity in mouse colon region.	
15	Stomach	-By qrtPCR and immunohistochemistry showed the expression of FFAR3 in villi and microvilli of gastric brush cells of mice stomach.	[2,6,183]
16	Enteric mucosal and submucosal cholinergic neurons of rat	-Suppresses carbachol (CCh)- or luminal propionate-induced Cl^- secretion influenced by TTX, hexamethonium and MQC through nicotinic ACh receptor activation. -SCFA-FFAR3 pathway responsible for anti-secretory function inhibited through cholinergic neural reflexes. -Pretreatment with serosal PTX along with MQC application restored the CCh response indicating the FFAR3 anti-secretory effect is mediated through $G_{i/o}$ pathway in rat proximal colon.	[200]
17	Adipocytes	-A mixture of SCFA reduces plasma FFA in DIO mice along with beige adipogenesis marker. -Increase in adipose tissues with reduction in colon size. -Reduction in *Firmicutes: Bacteroidetes* ratio. -Reduces body weight by increasing mitochondrial biogenesis and reducing chronic inflammation.	[18,202]
18	Lungs	-Expressed in the mice lungs. -Propionate minimize allergy airway inflammation in mice lungs mediated through FFAR3.	[5]
19	Duodenal I-cells	-The receptor senses the circulating SCFA in plasma to modulate I-cell functions. -But unlike the LCFA, SCFAs are not involved in the cholecystokinin synthesis from duodenal I-cells.	[225]

FFAR3: Free fatty acid receptor 3; GLP-1: Glucagon-like peptide 1; PYY: Peptide YY; CCK: Cholecystokinin; GIP: Glucose-dependent insulinotropic peptide; IGN: Intestinal Gluconeogenesis; TA: Tiglic acid; MA: Methylcyclopropanecarboxylic acid; hMADS: human multipotent adipose tissue-derived stem cells; PTX: Pertussis toxin; HSL: Hormone-sensitive lipase; Isc: Short-circuit currents; TTX: Tetrodotoxin; VIP: Vasoactive intestinal polypeptide; IRES: Internal ribosomal entry site; Compound 4: (N-(2,5-dichlorophenyl)-4-(furan2-yl)-2-methyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide); SNS: Sympathetic nervous system; SCG: Superior cervical ganglia; CSMG: Celiac-mesenteric Ganglia; BHB: β-hydroxybutyrate; NE: Norepinephrine; PLC: Phospholipase C; MEK: Methyl ethyl ketone; siRNA: Small interfering ribonucleic acid; PBN: Parabrachial nucleus; DVC: Dorsal vagal complex; PVN: Paraventricular nucleus; LH: Lateral hypothalamus; ARC:

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

Arcuate nucleus; KO: Knock-out; TTX: Tetrodotoxin; ACh: Acetylcholinergic; CGRP: Calcitonin gene related peptide; CCh: Carbachol; MQC: N-[2-methylphenyl]-[4-furan-3-yl]-2-methyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide; FFA: Free fatty acid; LCFA: Long chain fatty acid

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

ABBREVIATIONS USED IN THIS PAPER:

Abbreviation:

SCFAs: Short chain fatty acids; FFAR2: Free-fatty acid receptor 2; FFAR3: Free-fatty acid receptor 3; GPCRs: G-coupled protein receptors; T1D: Type-1 diabetes; T2D: Type-2 diabetes; IBD: Inflammatory bowel disease; TM: Transmembrane; PBMC: Peripheral blood mononuclear cells; PMNs: Polymorphonuclear cells; DCs: Dendritic cells; WAT: White adipose tissue; BAT: Brown adipose tissue; bMEC: bovine mammary epithelial cell line; SNS: Sympathetic nervous system; CNS: Central nervous system; CRC: Colorectal cancer; SCG: Superior cervical ganglia; CSMG: Celiac sympathetic-mesenteric ganglia; GI: gastrointestinal; AAs: Amino acids; Tyr: Tyrosine; Ile: Isoleucine; Arg: Arginine; Glu: Glutamate; EL: Extracellular loop; SCAs: Small carboxylic acids; Trp: Tryptophan; Gln: Glutamine; His: Histidine; Thr: Threonine; Phe: Phenylalanine; Leu: Leucine; SAR: Structure activity relationship; Å: Angstrom; SASA: Solvent accessible surface area; H: Hydrogen; PYY: Peptide YY; Cmp1: Compound 1; CFMB/Phenylacetamide 1: [(S)-2-(4-chlorophenyl)-3,3-dimethyl- N-(5-phenylthiazol-2-yl)butamide; AMG-7703: (2S)-2-(4-chlorophenyl)-3-methyl-N-(1,3-thiazol-2-yl)butanamide; Val: Valine; 4-CMTB: 2-(4-chlorophenyl)-3-methyl-N-(thiazole-2-yl)butanamide; 2CTAP: 4-((4-(2-chlorophenyl)thiazole-2-yl)amino)-4oxo-3-phenylbutanoic acid, BTI-A-404: [4-[4-(dimethylamino)phenyl]-N-(3,5-dimethylphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxamide]; BTI-A-292: [4-[4-(dimethylamino) phenyl]-N-(4,5-dimethylphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxamide]; CATPB: (S)-3-(2-(3-chlorophenyl)acetamido)-4-(4-(trifluoromethyl)phenyl)butanoic acid; GLPG0974: 4-[[(2R)-1-(1-benzothiophene-3-carbonyl)-2-methylazetidine-2-carbonyl]-[(3-chlorophenyl)methyl]amino]butanoic acid; Compound 1: 3-benzyl-4-(cyclopropyl-(4-(2,5-dichlorophenyl)thiazol-2-yl)amino)-4-oxobutanoic acid; Phenylacetamide 2/Compound 44: (S)-2-(4-chlorophenyl)-N-(5-fluorothiazol-2-yl)-3-methylbutanamide; Phenylacetamide 58: (S)-2-(4-chlorophenyl)-3,3-dimethyl-N-(5-phenylthiazol-2-yl)butanamide; 1-MCPC: 1-methylcyclopropane carboxylate; AR420626: N-(2,5-dichlorophenyl)-4-(furan-2-yl)-2-methyl-5-oxo-1,4,5,6,7,8-hexahydro-quinoline-3-carboxamide; CF_3-MQC: N-(2-methylphenyl)-4-[5-(2-trifluoromethoxy-phenyl)-furan-2-yl)-2-methyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide; MQC: N-[2-methylphenyl]-[4-furan-3-yl]-2-methyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide; DSS: Dextran sodium sulphate; LPS: Lipopolysaccharide; MAPK: Mitogen-activated protein kinase; Ig: Immunoglobulin; CRAMP: Cathelicidin related antimicrobial peptide; NFκB: Nuclear factor kappa-light-chain-enhancer of activated B cells; AMPK-α: 5' adenosine monophosphate-activated protein kinase; PI3K: Phosphatidylinositol 3-kinase; Akt: Protein Kinase B; ERK: Extracellular signal-regulated kinases; $FFAR2^{-/-}$: FFAR2 Knock-out; INFγ: Interferon gamma; PTX: Pertussis toxin; JNK: c-Jun N-terminal kinase; iNOS: Induced nitric oxide synthase; HUVEC: Human umbilical vein endothelial cells; HDACi: Histone deacetylases inhibitor; Treg: Regulatory T-cells; WT: Wild type; mTOR: The mammalian target of rapamycin; eNOS: Exhaled nitric oxide; TNF-α: Tumor Necrosis Factor alpha; GIT: Gastrointestinal tract; GLP-1: Glucagon-like peptide 1; POMC: Pro-opiomelanocortin; NPY: Neuropeptide Y; AgRP: Agouti-related peptide; qRTPCR: quantitative reverse transcription polymerase chain reaction; 5-HT: 5-hydroxytryptamine; HCO_3^-: Bicarbonate; cAMP: cyclic adenosine

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

monophosphate; FACS: Fluorescence-activated cell sorting; mRFP: monomeric red fluorescent protein; GIP: Gastric inhibitory polypeptide; RYGB: Roux-en-Y gastric bypass; M-cells: Myeloid cells; IECs: Intestinal epithelial cells; 5-HT: 5-hydroxytryptamine; IL-Interleukin; Tjp1: Tight junction protein 1; Ocln: Occludin; Cldn: Claudine; Muc: mucin; AMPK: AMP-activated protein kinase; HIF: hypoxia-inducible factor; DSS: Dextran sodium sulphate; TNBS: Trinitrobenzoic sulphonic acid; PNS: Peripheral nervous system; BBB: blood brain barrier; TH: tyrosine hydroxylase; PLC: Phospholipase C; nAChR: nicotinic acetylcholine receptor; Ca: Calcium; IP: Intraperitoneal; DIO: Diet induced obese; HFD: High fat diet; siRNA: small interfering ribonucleic acid; Peroxisome proliferator-activated receptor gamma: PPAR; hMADS: human multipotent adipose tissue-derived stem; GBC: Gall bladder cancer; GC: Gastric cancer; ISCs: Intestinal stem cells; NC: Normal chow; OGTT: Oral glucose tolerance test; IM-BAT: Immortalized brown adipocyte cell line; GSIS: Glucose-stimulated insulin secretion; DSS: Dextran sodium sulphate; DAI: daily activity index; Raf: rapidly accelerated fibrosarcoma.

Author Contributions: Conceptualization, project administration, supervision, funding acquisition, writing—review and editing, resources- H.Y.; methodology, writing-original draft preparation, investigation, S.P.M.; FFAR2/3 modeling and bioinformatics analyses- P.K., and feedback on manuscript- S.T. All authors have read and agreed to the published version of the manuscript.",

Funding: We are thankful for the support provided by National Institutes of Health grants- R01AG018915 and the Pepper Older Americans for Independence Center (P30AG21332), and the Department of Defense funding W81XWH-18-1-0118 and W81XWH-19-1-0236 (HY), as well funds and services provided from the Center for Diabetes, Obesity and Metabolism, Wake Forest Baptist Medical Center, and the National Center for Advancing Translational Sciences (NCATS), the National Institutes of Health-funded Wake Forest Clinical and Translational Science Institute (WF CTSI) through Grant Award Number UL1TR001420.

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

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

References

1. Nagpal, R.; Wang, S.; Ahmadi, S.; Hayes, J.; Gagliano, J.; Subashchandrabose, S.; Kitzman, D.W.; Becton, T.; Read, R.; Yadav, H. Human-origin probiotic cocktail increases short-chain fatty acid production via modulation of mice and human gut microbiome. *Scientific Reports* **2018**, *8*, 12649, doi:10.1038/s41598-018-30114-4.
2. Nohr, M.K.; Pedersen, M.H.; Gille, A.; Egerod, K.L.; Engelstoft, M.S.; Husted, A.S.; Sichlau, R.M.; Grunddal, K.V.; Poulsen, S.S.; Han, S., et al. GPR41/FFAR3 and GPR43/FFAR2 as cosensors for short-chain fatty acids in enteroendocrine cells vs FFAR3 in enteric neurons and FFAR2 in enteric leukocytes. *Endocrinology* **2013**, *154*, 3552-3564, doi:10.1210/en.2013-1142.
3. Samuel, B.S.; Shaito, A.; Motoike, T.; Rey, F.E.; Backhed, F.; Manchester, J.K.; Hammer, R.E.; Williams, S.C.; Crowley, J.; Yanagisawa, M., et al. Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. *Proceedings of the National Academy of Sciences of the United States of America* **2008**, *105*, 16767-16772, doi:10.1073/pnas.0808567105.
4. Shimizu, H.; Masujima, Y.; Ushiroda, C.; Mizushima, R.; Taira, S.; Ohue-Kitano, R.; Kimura, I. Dietary short-chain fatty acid intake improves the hepatic metabolic condition via FFAR3. *Sci Rep* **2019**, *9*, 16574, doi:10.1038/s41598-019-53242-x.
5. Trompette, A.; Gollwitzer, E.S.; Yadava, K.; Sichelstiel, A.K.; Sprenger, N.; Ngom-Bru, C.; Blanchard, C.; Junt, T.; Nicod, L.P.; Harris, N.L., et al. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nature medicine* **2014**, *20*, 159-166, doi:10.1038/nm.3444.
6. Eberle, J.A.; Widmayer, P.; Breer, H. Receptors for short-chain fatty acids in brush cells at the "gastric groove". *Front Physiol* **2014**, *5*, 152, doi:10.3389/fphys.2014.00152.
7. D'Souza, W.N.; Douangpanya, J.; Mu, S.; Jaeckel, P.; Zhang, M.; Maxwell, J.R.; Rottman, J.B.; Labitzke, K.; Willee, A.; Beckmann, H., et al. Differing roles for short chain fatty acids and GPR43 agonism in the regulation of intestinal barrier function and immune responses. *PLoS One* **2017**, *12*, e0180190, doi:10.1371/journal.pone.0180190.
8. Erny, D.; Hrabe de Angelis, A.L.; Jaitin, D.; Wieghofer, P.; Staszewski, O.; David, E.; Keren-Shaul, H.; Mahlakoiv, T.; Jakobshagen, K.; Buch, T., et al. Host microbiota constantly control maturation and function of microglia in the CNS. *Nature neuroscience* **2015**, *18*, 965-977, doi:10.1038/nn.4030.
9. Montalvany-Antonucci, C.C.; Duffles, L.F.; de Arruda, J.A.A.; Zicker, M.C.; de Oliveira, S.; Macari, S.; Garlet, G.P.; Madeira, M.F.M.; Fukada, S.Y.; Andrade, I., Jr., et al. Short-chain fatty acids and FFAR2 as suppressors of bone resorption. *Bone* **2019**, *125*, 112-121, doi:10.1016/j.bone.2019.05.016.
10. Tolhurst, G.; Heffron, H.; Lam, Y.S.; Parker, H.E.; Habib, A.M.; Diakogiannaki, E.; Cameron, J.; Grosse, J.; Reimann, F.; Gribble, F.M. Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. *Diabetes* **2012**, *61*, 364-371, doi:10.2337/db11-1019.
11. Friedrichs, P.; Sauerwein, H.; Huber, K.; Locher, L.F.; Rehage, J.; Meyer, U.; Danicke, S.; Kuhla, B.; Mielenz, M. Expression of metabolic sensing receptors in adipose tissues of periparturient dairy cows with differing extent of negative energy balance. *Animal* **2016**, *10*, 623-632, doi:10.1017/S175173111500227X.
12. Li, G.; Su, H.; Zhou, Z.; Yao, W. Identification of the porcine G protein-coupled receptor 41 and 43 genes and their expression pattern in different tissues and development stages. *PLoS One* **2014**, *9*, e97342, doi:10.1371/journal.pone.0097342.
13. Ivan, J.; Major, E.; Sipos, A.; Kovacs, K.; Horvath, D.; Tamas, I.; Bay, P.; Dombradi, V.; Lontay, B. The Short-Chain Fatty Acid Propionate Inhibits Adipogenic Differentiation of Human Chorion-Derived

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

Mesenchymal Stem Cells Through the Free Fatty Acid Receptor 2. *Stem Cells Dev* **2017**, *26*, 1724-1733, doi:10.1089/scd.2017.0035.

14. Jocken, J.W.E.; Gonzalez Hernandez, M.A.; Hoebers, N.T.H.; van der Beek, C.M.; Essers, Y.P.G.; Blaak, E.E.; Canfora, E.E. Short-Chain Fatty Acids Differentially Affect Intracellular Lipolysis in a Human White Adipocyte Model. *Front Endocrinol (Lausanne)* **2017**, *8*, 372, doi:10.3389/fendo.2017.00372.
15. Veprik, A.; Laufer, D.; Weiss, S.; Rubins, N.; Walker, M.D. GPR41 modulates insulin secretion and gene expression in pancreatic beta-cells and modifies metabolic homeostasis in fed and fasting states. *Faseb j* **2016**, *30*, 3860-3869, doi:10.1096/fj.201500030R.
16. den Besten, G.; Lange, K.; Havinga, R.; van Dijk, T.H.; Gerding, A.; van Eunen, K.; Muller, M.; Groen, A.K.; Hooiveld, G.J.; Bakker, B.M., et al. Gut-derived short-chain fatty acids are vividly assimilated into host carbohydrates and lipids. *Am J Physiol Gastrointest Liver Physiol* **2013**, *305*, G900-910, doi:10.1152/ajpgi.00265.2013.
17. Bindels, L.B.; Porporato, P.; Dewulf, E.M.; Verrax, J.; Neyrinck, A.M.; Martin, J.C.; Scott, K.P.; Buc Calderon, P.; Feron, O.; Muccioli, G.G., et al. Gut microbiota-derived propionate reduces cancer cell proliferation in the liver. *British journal of cancer* **2012**, *107*, 1337-1344, doi:10.1038/bjc.2012.409.
18. Lu, Y.; Fan, C.; Li, P.; Lu, Y.; Chang, X.; Qi, K. Short Chain Fatty Acids Prevent High-fat-diet-induced Obesity in Mice by Regulating G Protein-coupled Receptors and Gut Microbiota. *Sci Rep* **2016**, *6*, 37589, doi:10.1038/srep37589.
19. Pluznick, J.L.; Protzko, R.J.; Gevorgyan, H.; Peterlin, Z.; Sipos, A.; Han, J.; Brunet, I.; Wan, L.X.; Rey, F.; Wang, T., et al. Olfactory receptor responding to gut microbiota-derived signals plays a role in renin secretion and blood pressure regulation. *Proceedings of the National Academy of Sciences of the United States of America* **2013**, *110*, 4410-4415, doi:10.1073/pnas.1215927110.
20. Bindels, L.B.; Porporato, P.E.; Ducastel, S.; Sboarina, M.; Neyrinck, A.M.; Dewulf, E.M.; Feron, O.; Lestavel, S.; Cani, P.D.; Staels, B., et al. Ffar2 expression regulates leukaemic cell growth in vivo. *British journal of cancer* **2017**, *117*, 1336-1340, doi:10.1038/bjc.2017.307.
21. Ang, Z.; Er, J.Z.; Tan, N.S.; Lu, J.; Liou, Y.C.; Grosse, J.; Ding, J.L. Human and mouse monocytes display distinct signalling and cytokine profiles upon stimulation with FFAR2/FFAR3 short-chain fatty acid receptor agonists. *Sci Rep* **2016**, *6*, 34145, doi:10.1038/srep34145.
22. Kimura, I.; Inoue, D.; Maeda, T.; Hara, T.; Ichimura, A.; Miyauchi, S.; Kobayashi, M.; Hirasawa, A.; Tsujimoto, G. Short-chain fatty acids and ketones directly regulate sympathetic nervous system via G protein-coupled receptor 41 (GPR41). *Proceedings of the National Academy of Sciences of the United States of America* **2011**, *108*, 8030-8035, doi:10.1073/pnas.1016088108.
23. Brooks, L.; Viardot, A.; Tsakmaki, A.; Stolarczyk, E.; Howard, J.K.; Cani, P.D.; Everard, A.; Sleeth, M.L.; Psichas, A.; Anastasovskaj, J., et al. Fermentable carbohydrate stimulates FFAR2-dependent colonic PYY cell expansion to increase satiety. *Mol Metab* **2017**, *6*, 48-60, doi:10.1016/j.molmet.2016.10.011.
24. Villa, S.R.; Priyadarshini, M.; Fuller, M.H.; Bhardwaj, T.; Brodsky, M.R.; Angueira, A.R.; Mosser, R.E.; Carboneau, B.A.; Tersey, S.A.; Mancebo, H., et al. Loss of Free Fatty Acid Receptor 2 leads to impaired islet mass and beta cell survival. *Sci Rep* **2016**, *6*, 28159, doi:10.1038/srep28159.
25. Priyadarshini, M.; Villa, S.R.; Fuller, M.; Wicksteed, B.; Mackay, C.R.; Alquier, T.; Poitout, V.; Mancebo, H.; Mirmira, R.G.; Gilchrist, A., et al. An Acetate-Specific GPCR, FFAR2, Regulates Insulin Secretion. *Mol Endocrinol* **2015**, *29*, 1055-1066, doi:10.1210/me.2015-1007.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

26. Remely, M.; Aumueller, E.; Merold, C.; Dworzak, S.; Hippe, B.; Zanner, J.; Pointner, A.; Brath, H.; Haslberger, A.G. Effects of short chain fatty acid producing bacteria on epigenetic regulation of FFAR3 in type 2 diabetes and obesity. *Gene* **2014**, *537*, 85-92, doi:10.1016/j.gene.2013.11.081.
27. Tang, C.; Ahmed, K.; Gille, A.; Lu, S.; Grone, H.J.; Tunaru, S.; Offermanns, S. Loss of FFA2 and FFA3 increases insulin secretion and improves glucose tolerance in type 2 diabetes. *Nat Med* **2015**, *21*, 173-177, doi:10.1038/nm.3779.
28. Shi, G.; Sun, C.; Gu, W.; Yang, M.; Zhang, X.; Zhai, N.; Lu, Y.; Zhang, Z.; Shou, P.; Zhang, Z., et al. Free fatty acid receptor 2, a candidate target for type 1 diabetes, induces cell apoptosis through ERK signaling. *Journal of molecular endocrinology* **2014**, *53*, 367-380, doi:10.1530/jme-14-0065.
29. Kimura, I.; Ozawa, K.; Inoue, D.; Imamura, T.; Kimura, K.; Maeda, T.; Terasawa, K.; Kashihara, D.; Hirano, K.; Tani, T., et al. The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. *Nat Commun* **2013**, *4*, 1829, doi:10.1038/ncomms2852.
30. Ge, H.; Li, X.; Weiszmann, J.; Wang, P.; Baribault, H.; Chen, J.L.; Tian, H.; Li, Y. Activation of G protein-coupled receptor 43 in adipocytes leads to inhibition of lipolysis and suppression of plasma free fatty acids. *Endocrinology* **2008**, *149*, 4519-4526, doi:10.1210/en.2008-0059.
31. McNelis, J.C.; Lee, Y.S.; Mayoral, R.; van der Kant, R.; Johnson, A.M.; Wollam, J.; Olefsky, J.M. GPR43 Potentiates beta-Cell Function in Obesity. *Diabetes* **2015**, *64*, 3203-3217, doi:10.2337/db14-1938.
32. Bjursell, M.; Admyre, T.; Goransson, M.; Marley, A.E.; Smith, D.M.; Oscarsson, J.; Bohlooly, Y.M. Improved glucose control and reduced body fat mass in free fatty acid receptor 2-deficient mice fed a high-fat diet. *Am J Physiol Endocrinol Metab* **2011**, *300*, E211-220, doi:10.1152/ajpendo.00229.2010.
33. Tang, Y.; Chen, Y.; Jiang, H.; Robbins, G.T.; Nie, D. G-protein-coupled receptor for short-chain fatty acids suppresses colon cancer. *Int J Cancer* **2011**, *128*, 847-856, doi:10.1002/ijc.25638.
34. Maslowski, K.M.; Vieira, A.T.; Ng, A.; Kranich, J.; Sierro, F.; Yu, D.; Schilter, H.C.; Rolph, M.S.; Mackay, F.; Artis, D., et al. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature* **2009**, *461*, 1282-1286, doi:10.1038/nature08530.
35. Zhao, M.; Zhu, W.; Gong, J.; Zuo, L.; Zhao, J.; Sun, J.; Li, N.; Li, J. Dietary Fiber Intake is Associated with Increased Colonic Mucosal GPR43+ Polymorphonuclear Infiltration in Active Crohn's Disease. *Nutrients* **2015**, *7*, 5327-5346, doi:10.3390/nu7075223.
36. Natarajan, N.; Hori, D.; Flavahan, S.; Steppan, J.; Flavahan, N.A.; Berkowitz, D.E.; Pluznick, J.L. Microbial short chain fatty acid metabolites lower blood pressure via endothelial G protein-coupled receptor 41. *Physiological genomics* **2016**, *48*, 826-834, doi:10.1152/physiolgenomics.00089.2016.
37. Ruan, J.; Meng, H.; Wang, X.; Chen, W.; Tian, X.; Meng, F. Low Expression of FFAR2 in Peripheral White Blood Cells May Be a Genetic Marker for Early Diagnosis of Acute Myocardial Infarction. *Cardiology Research and Practice* **2020**, *2020*, 1-8, doi:10.1155/2020/3108124.
38. Vieira, A.T.; Macia, L.; Galvao, I.; Martins, F.S.; Canesso, M.C.; Amaral, F.A.; Garcia, C.C.; Maslowski, K.M.; De Leon, E.; Shim, D., et al. A Role for Gut Microbiota and the Metabolite-Sensing Receptor GPR43 in a Murine Model of Gout. *Arthritis Rheumatol* **2015**, *67*, 1646-1656, doi:10.1002/art.39107.
39. Halnes, I.; Baines, K.J.; Berthon, B.S.; MacDonald-Wicks, L.K.; Gibson, P.G.; Wood, L.G. Soluble Fibre Meal Challenge Reduces Airway Inflammation and Expression of GPR43 and GPR41 in Asthma. *Nutrients* **2017**, *9*, doi:10.3390/nu9010057.
40. Eckalbar, W.L.; Erle, D.J. Singling out Th2 cells in eosinophilic esophagitis. *J Clin Invest* **2019**, *129*, 1830-1832, doi:10.1172/JCI128479.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

41. Masui, R.; Sasaki, M.; Funaki, Y.; Ogasawara, N.; Mizuno, M.; Iida, A.; Izawa, S.; Kondo, Y.; Ito, Y.; Tamura, Y., et al. G protein-coupled receptor 43 moderates gut inflammation through cytokine regulation from mononuclear cells. *Inflamm Bowel Dis* **2013**, *19*, 2848-2856, doi:10.1097/01.MIB.0000435444.14860.ea.

42. Sina, C.; Gavrilova, O.; Forster, M.; Till, A.; Derer, S.; Hildebrand, F.; Raabe, B.; Chalaris, A.; Scheller, J.; Rehmann, A., et al. G protein-coupled receptor 43 is essential for neutrophil recruitment during intestinal inflammation. *J Immunol* **2009**, *183*, 7514-7522, doi:10.4049/jimmunol.0900063.

43. Kim, M.H.; Kang, S.G.; Park, J.H.; Yanagisawa, M.; Kim, C.H. Short-chain fatty acids activate GPR41 and GPR43 on intestinal epithelial cells to promote inflammatory responses in mice. *Gastroenterology* **2013**, *145*, 396-406 e391-310, doi:10.1053/j.gastro.2013.04.056.

44. Macia, L.; Tan, J.; Vieira, A.T.; Leach, K.; Stanley, D.; Luong, S.; Maruya, M.; Ian McKenzie, C.; Hijikata, A.; Wong, C., et al. Metabolite-sensing receptors GPR43 and GPR109A facilitate dietary fibre-induced gut homeostasis through regulation of the inflammasome. *Nat Commun* **2015**, *6*, 6734, doi:10.1038/ncomms7734.

45. Tikhonova, I.G. Application of GPCR Structures for Modelling of Free Fatty Acid Receptors. *Handbook of experimental pharmacology* **2017**, *236*, 57-77, doi:10.1007/164_2016_52.

46. Wang, G.; Jiang, L.; Wang, J.; Zhang, J.; Kong, F.; Li, Q.; Yan, Y.; Huang, S.; Zhao, Y.; Liang, L., et al. The G Protein-Coupled Receptor FFAR2 Promotes Internalization during Influenza A Virus Entry. *J Virol* **2020**, *94*, doi:10.1128/JVI.01707-19.

47. Lagerstrom, M.C.; Schioth, H.B. Structural diversity of G protein-coupled receptors and significance for drug discovery. *Nat Rev Drug Discov* **2008**, *7*, 339-357, doi:10.1038/nrd2518.

48. Venkatakrishnan, A.J.; Deupi, X.; Lebon, G.; Tate, C.G.; Schertler, G.F.; Babu, M.M. Molecular signatures of G-protein-coupled receptors. *Nature* **2013**, *494*, 185-194, doi:10.1038/nature11896.

49. Ohira, H.; Fujioka, Y.; Katagiri, C.; Mamoto, R.; Aoyama-Ishikawa, M.; Amako, K.; Izumi, Y.; Nishiumi, S.; Yoshida, M.; Usami, M., et al. Butyrate attenuates inflammation and lipolysis generated by the interaction of adipocytes and macrophages. *Journal of atherosclerosis and thrombosis* **2013**, *20*, 425-442, doi:10.5551/jat.15065.

50. Priyadarshini, M.; Layden, B.T. FFAR3 modulates insulin secretion and global gene expression in mouse islets. *Islets* **2015**, *7*, e1045182, doi:10.1080/19382014.2015.1045182.

51. Brown, A.J.; Goldsworthy, S.M.; Barnes, A.A.; Eilert, M.M.; Tcheang, L.; Daniels, D.; Muir, A.I.; Wigglesworth, M.J.; Kinghorn, I.; Fraser, N.J., et al. The Orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *J Biol Chem* **2003**, *278*, 11312-11319, doi:10.1074/jbc.M211609200.

52. Le Poul, E.; Loison, C.; Struyf, S.; Springael, J.Y.; Lannoy, V.; Decobecq, M.E.; Brezillon, S.; Dupriez, V.; Vassart, G.; Van Damme, J., et al. Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. *J Biol Chem* **2003**, *278*, 25481-25489, doi:10.1074/jbc.M301403200.

53. Nilsson, N.E.; Kotarsky, K.; Owman, C.; Olde, B. Identification of a free fatty acid receptor, FFA2R, expressed on leukocytes and activated by short-chain fatty acids. *Biochemical and biophysical research communications* **2003**, *303*, 1047-1052.

54. Cox, M.A.; Jackson, J.; Stanton, M.; Rojas-Triana, A.; Bober, L.; Laverty, M.; Yang, X.; Zhu, F.; Liu, J.; Wang, S., et al. Short-chain fatty acids act as antiinflammatory mediators by regulating prostaglandin E(2) and cytokines. *World J Gastroenterol* **2009**, *15*, 5549-5557, doi:10.3748/wjg.15.5549.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

55. Carr, Z.J.; Van De Louw, A.; Fehr, G.; Li, J.D.; Kunselman, A.; Ruiz-Velasco, V. Increased whole blood FFA2/GPR43 receptor expression is associated with increased 30-day survival in patients with sepsis. *BMC Res Notes* **2018**, *11*, 41, doi:10.1186/s13104-018-3165-4.

56. Liu, D.; Costanzo, A.; Evans, M.D.M.; Archer, N.S.; Nowson, C.; Duesing, K.; Keast, R. Expression of the candidate fat taste receptors in human fungiform papillae and the association with fat taste function. *Br J Nutr* **2018**, *120*, 64-73, doi:10.1017/S0007114518001265.

57. Kirkling, M.E.; Cytlak, U.; Lau, C.M.; Lewis, K.L.; Resteu, A.; Khodadadi-Jamayran, A.; Siebel, C.W.; Salmon, H.; Merad, M.; Tsirigos, A., et al. Notch Signaling Facilitates In Vitro Generation of Cross-Presenting Classical Dendritic Cells. *Cell reports* **2018**, *23*, 3658-3672.e3656, doi:10.1016/j.celrep.2018.05.068.

58. Pingitore, A.; Gonzalez-Abuin, N.; Ruz-Maldonado, I.; Huang, G.C.; Frost, G.; Persaud, S.J. Short chain fatty acids stimulate insulin secretion and reduce apoptosis in mouse and human islets in vitro: Role of free fatty acid receptor 2. *Diabetes, obesity & metabolism* **2019**, *21*, 330-339, doi:10.1111/dom.13529.

59. Pingitore, A.; Chambers, E.S.; Hill, T.; Maldonado, I.R.; Liu, B.; Bewick, G.; Morrison, D.J.; Preston, T.; Wallis, G.A.; Tedford, C., et al. The diet-derived short chain fatty acid propionate improves beta-cell function in humans and stimulates insulin secretion from human islets in vitro. *Diabetes, obesity & metabolism* **2017**, *19*, 257-265, doi:10.1111/dom.12811.

60. Voltolini, C.; Battersby, S.; Etherington, S.L.; Petraglia, F.; Norman, J.E.; Jabbour, H.N. A novel antiinflammatory role for the short-chain fatty acids in human labor. *Endocrinology* **2012**, *153*, 395-403, doi:10.1210/en.2011-1457.

61. Karaki, S.; Tazoe, H.; Hayashi, H.; Kashiwabara, H.; Tooyama, K.; Suzuki, Y.; Kuwahara, A. Expression of the short-chain fatty acid receptor, GPR43, in the human colon. *J Mol Histol* **2008**, *39*, 135-142, doi:10.1007/s10735-007-9145-y.

62. Hoyles, L.; Snelling, T.; Umlai, U.K.; Nicholson, J.K.; Carding, S.R.; Glen, R.C.; McArthur, S. Microbiome-host systems interactions: protective effects of propionate upon the blood-brain barrier. *Microbiome* **2018**, *6*, 55, doi:10.1186/s40168-018-0439-y.

63. Yonezawa, T.; Kobayashi, Y.; Obara, Y. Short-chain fatty acids induce acute phosphorylation of the p38 mitogen-activated protein kinase/heat shock protein 27 pathway via GPR43 in the MCF-7 human breast cancer cell line. *Cellular signalling* **2007**, *19*, 185-193, doi:10.1016/j.cellsig.2006.06.004.

64. Tazoe, H.; Otomo, Y.; Kaji, I.; Tanaka, R.; Karaki, S.I.; Kuwahara, A. Roles of short-chain fatty acids receptors, GPR41 and GPR43 on colonic functions. *Journal of physiology and pharmacology : an official journal of the Polish Physiological Society* **2008**, *59 Suppl 2*, 251-262.

65. Layden, B.T.; Angueira, A.R.; Brodsky, M.; Durai, V.; Lowe, W.L., Jr. Short chain fatty acids and their receptors: new metabolic targets. *Translational research : the journal of laboratory and clinical medicine* **2013**, *161*, 131-140, doi:10.1016/j.trsl.2012.10.007.

66. Li, L.; Hua, Y.; Ren, J. Short-chain fatty acid propionate alleviates Akt2 knockout-induced myocardial contractile dysfunction. *Exp Diabetes Res* **2012**, *2012*, 851717, doi:10.1155/2012/851717.

67. Senga, T.; Iwamoto, S.; Yoshida, T.; Yokota, T.; Adachi, K.; Azuma, E.; Hamaguchi, M.; Iwamoto, T. LSSIG is a novel murine leukocyte-specific GPCR that is induced by the activation of STAT3. *Blood* **2003**, *101*, 1185-1187, doi:10.1182/blood-2002-06-1881.

68. Smith, P.M.; Howitt, M.R.; Panikov, N.; Michaud, M.; Gallini, C.A.; Bohlooly, Y.M.; Glickman, J.N.; Garrett, W.S. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science (New York, N.Y.)* **2013**, *341*, 569-573, doi:10.1126/science.1241165.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

69. Agus, A.; Denizot, J.; Thevenot, J.; Martinez-Medina, M.; Massier, S.; Sauvanet, P.; Bernalier-Donadille, A.; Denis, S.; Hofman, P.; Bonnet, R., et al. Western diet induces a shift in microbiota composition enhancing susceptibility to Adherent-Invasive E. coli infection and intestinal inflammation. *Sci Rep* **2016**, *6*, 19032, doi:10.1038/srep19032.

70. den Besten, G.; Gerding, A.; van Dijk, T.H.; Ciapaite, J.; Bleeker, A.; van Eunen, K.; Havinga, R.; Groen, A.K.; Reijngoud, D.J.; Bakker, B.M. Protection against the Metabolic Syndrome by Guar Gum-Derived Short-Chain Fatty Acids Depends on Peroxisome Proliferator-Activated Receptor gamma and Glucagon-Like Peptide-1. *PLoS One* **2015**, *10*, e0136364, doi:10.1371/journal.pone.0136364.

71. Kebede, M.A.; Alquier, T.; Latour, M.G.; Poitout, V. Lipid receptors and islet function: therapeutic implications? *Diabetes, obesity & metabolism* **2009**, *11 Suppl 4*, 10-20, doi:10.1111/j.1463-1326.2009.01114.x.

72. Bahar Halpern, K.; Veprik, A.; Rubins, N.; Naaman, O.; Walker, M.D. GPR41 gene expression is mediated by internal ribosome entry site (IRES)-dependent translation of bicistronic mRNA encoding GPR40 and GPR41 proteins. *J Biol Chem* **2012**, *287*, 20154-20163, doi:10.1074/jbc.M112.358887.

73. Zaibi, M.S.; Stocker, C.J.; O'Dowd, J.; Davies, A.; Bellahcene, M.; Cawthorne, M.A.; Brown, A.J.; Smith, D.M.; Arch, J.R. Roles of GPR41 and GPR43 in leptin secretory responses of murine adipocytes to short chain fatty acids. *FEBS Lett* **2010**, *584*, 2381-2386, doi:10.1016/j.febslet.2010.04.027.

74. Hong, Y.H.; Nishimura, Y.; Hishikawa, D.; Tsuzuki, H.; Miyahara, H.; Gotoh, C.; Choi, K.C.; Feng, D.D.; Chen, C.; Lee, H.G., et al. Acetate and propionate short chain fatty acids stimulate adipogenesis via GPCR43. *Endocrinology* **2005**, *146*, 5092-5099, doi:10.1210/en.2005-0545.

75. Karaki, S.; Mitsui, R.; Hayashi, H.; Kato, I.; Sugiya, H.; Iwanaga, T.; Furness, J.B.; Kuwahara, A. Short-chain fatty acid receptor, GPR43, is expressed by enteroendocrine cells and mucosal mast cells in rat intestine. *Cell Tissue Res* **2006**, *324*, 353-360, doi:10.1007/s00441-005-0140-x.

76. Dass, N.B.; John, A.K.; Bassil, A.K.; Crumbley, C.W.; Shehee, W.R.; Maurio, F.P.; Moore, G.B.; Taylor, C.M.; Sanger, G.J. The relationship between the effects of short-chain fatty acids on intestinal motility in vitro and GPR43 receptor activation. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society* **2007**, *19*, 66-74, doi:10.1111/j.1365-2982.2006.00853.x.

77. Holota, Y.; Dovbynchuk, T.; Kaji, I.; Vareniuk, I.; Dzyubenko, N.; Chervinska, T.; Zakordonets, L.; Stetska, V.; Ostapchenko, L.; Serhiychuk, T., et al. The long-term consequences of antibiotic therapy: Role of colonic short-chain fatty acids (SCFA) system and intestinal barrier integrity. *PLoS One* **2019**, *14*, e0220642, doi:10.1371/journal.pone.0220642.

78. Wang, A.; Gu, Z.; Heid, B.; Akers, R.M.; Jiang, H. Identification and characterization of the bovine G protein-coupled receptor GPR41 and GPR43 genes. *Journal of dairy science* **2009**, *92*, 2696-2705, doi:10.3168/jds.2009-2037.

79. Aguinaga Casanas, M.A.; Schaff, C.T.; Albrecht, E.; Hammon, H.M.; Kuhla, B.; Rontgen, M.; Nurnberg, G.; Mielenz, M. Short communication: Free fatty acid receptors FFAR1 and FFAR2 during the peripartal period in liver of dairy cows grouped by their postpartum plasma beta-hydroxybutyrate concentrations. *Journal of dairy science* **2017**, *100*, 3287-3292, doi:10.3168/jds.2016-11021.

80. Dias, A.L.G.; Freitas, J.A.; Micai, B.; Azevedo, R.A.; Greco, L.F.; Santos, J.E.P. Effect of supplemental yeast culture and dietary starch content on rumen fermentation and digestion in dairy cows. *Journal of dairy science* **2018**, *101*, 201-221, doi:10.3168/jds.2017-13241.

81. Hosseini, A.; Behrendt, C.; Regenhard, P.; Sauerwein, H.; Mielenz, M. Differential effects of propionate or beta-hydroxybutyrate on genes related to energy balance and insulin sensitivity in bovine white

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

adipose tissue explants from a subcutaneous and a visceral depot. *Journal of animal physiology and animal nutrition* **2012**, *96*, 570-580, doi:10.1111/j.1439-0396.2011.01180.x.

82. Choi, S.H.; Chung, K.Y.; Johnson, B.J.; Go, G.W.; Kim, K.H.; Choi, C.W.; Smith, S.B. Co-culture of bovine muscle satellite cells with preadipocytes increases PPARgamma and C/EBPbeta gene expression in differentiated myoblasts and increases GPR43 gene expression in adipocytes. *The Journal of nutritional biochemistry* **2013**, *24*, 539-543, doi:10.1016/j.jnutbio.2012.01.015.
83. Lemor, A.; Hosseini, A.; Sauerwein, H.; Mielenz, M. Transition period-related changes in the abundance of the mRNAs of adiponectin and its receptors, of visfatin, and of fatty acid binding receptors in adipose tissue of high-yielding dairy cows. *Domest Anim Endocrinol* **2009**, *37*, 37-44, doi:10.1016/j.domaniend.2009.01.004.
84. Yonezawa, T.; Haga, S.; Kobayashi, Y.; Katoh, K.; Obara, Y. Short-chain fatty acid signaling pathways in bovine mammary epithelial cells. *Regul Pept* **2009**, *153*, 30-36, doi:10.1016/j.regpep.2008.11.012.
85. Li, G.; Yao, W.; Jiang, H. Short-chain fatty acids enhance adipocyte differentiation in the stromal vascular fraction of porcine adipose tissue. *The Journal of nutrition* **2014**, *144*, 1887-1895, doi:10.3945/jn.114.198531.
86. Yan, H.; Ajuwon, K.M. Mechanism of Butyrate Stimulation of Triglyceride Storage and Adipokine Expression during Adipogenic Differentiation of Porcine Stromovascular Cells. *PLoS One* **2015**, *10*, e0145940, doi:10.1371/journal.pone.0145940.
87. Haenen, D.; Zhang, J.; Souza da Silva, C.; Bosch, G.; van der Meer, I.M.; van Arkel, J.; van den Borne, J.J.; Perez Gutierrez, O.; Smidt, H.; Kemp, B., et al. A diet high in resistant starch modulates microbiota composition, SCFA concentrations, and gene expression in pig intestine. *The Journal of nutrition* **2013**, *143*, 274-283, doi:10.3945/jn.112.169672.
88. Ladeira, M.M.; Schoonmaker, J.P.; Swanson, K.C.; Duckett, S.K.; Gionbelli, M.P.; Rodrigues, L.M.; Teixeira, P.D. Review: Nutrigenomics of marbling and fatty acid profile in ruminant meat. *Animal* **2018**, *12*, s282-s294, doi:10.1017/s1751731118001933.
89. Gossner, A.; Wilkie, H.; Joshi, A.; Hopkins, J. Exploring the abomasal lymph node transcriptome for genes associated with resistance to the sheep nematode Teladorsagia circumcincta. *Vet Res* **2013**, *44*, 68, doi:10.1186/1297-9716-44-68.
90. Fu, C.Y.; Liu, L.; Gao, Q.; Sui, X.Y.; Li, F.C. Cloning, molecular characterization, and spatial and developmental expression analysis of GPR41 and GPR43 genes in New Zealand rabbits. *Animal* **2017**, *11*, 1798-1806, doi:10.1017/S175173111700043X.
91. Meslin, C.; Desert, C.; Callebaut, I.; Djari, A.; Klopp, C.; Pitel, F.; Leroux, S.; Martin, P.; Froment, P.; Guilbert, E., et al. Expanding Duplication of Free Fatty Acid Receptor-2 (GPR43) Genes in the Chicken Genome. *Genome Biol Evol* **2015**, *7*, 1332-1348, doi:10.1093/gbe/evv072.
92. Wei, Q.; Ramsey, S.A.; Larson, M.K.; Berlow, N.E.; Ochola, D.; Shiprack, C.; Kashyap, A.; Seguin, B.; Keller, C.; Lohr, C.V. Elucidating the transcriptional program of feline injection-site sarcoma using a cross-species mRNA-sequencing approach. *BMC Cancer* **2019**, *19*, 311, doi:10.1186/s12885-019-5501-z.
93. Read, J.E.; Cabrera-Sharp, V.; Offord, V.; Mirczuk, S.M.; Allen, S.P.; Fowkes, R.C.; de Mestre, A.M. Dynamic changes in gene expression and signalling during trophoblast development in the horse. *Reproduction (Cambridge, England)* **2018**, 10.1530/rep-18-0270, doi:10.1530/rep-18-0270.
94. Tang, X.L.; Wang, Y.; Li, D.L.; Luo, J.; Liu, M.Y. Orphan G protein-coupled receptors (GPCRs): biological functions and potential drug targets. *Acta Pharmacol Sin* **2012**, *33*, 363-371, doi:10.1038/aps.2011.210.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

95. Puhl, H.L., 3rd; Won, Y.J.; Lu, V.B.; Ikeda, S.R. Human GPR42 is a transcribed multisite variant that exhibits copy number polymorphism and is functional when heterologously expressed. *Sci Rep* **2015**, *5*, 12880, doi:10.1038/srep12880.

96. Layden, B.T.; Durai, V.; Newman, M.V.; Marinelarena, A.M.; Ahn, C.W.; Feng, G.; Lin, S.; Zhang, X.; Kaufman, D.B.; Jafari, N., et al. Regulation of pancreatic islet gene expression in mouse islets by pregnancy. *J Endocrinol* **2010**, *207*, 265-279, doi:10.1677/JOE-10-0298.

97. Regard, J.B.; Kataoka, H.; Cano, D.A.; Camerer, E.; Yin, L.; Zheng, Y.W.; Scanlan, T.S.; Hebrok, M.; Coughlin, S.R. Probing cell type-specific functions of Gi in vivo identifies GPCR regulators of insulin secretion. *J Clin Invest* **2007**, *117*, 4034-4043, doi:10.1172/JCI32994.

98. Xiong, Y.; Miyamoto, N.; Shibata, K.; Valasek, M.A.; Motoike, T.; Kedzierski, R.M.; Yanagisawa, M. Short-chain fatty acids stimulate leptin production in adipocytes through the G protein-coupled receptor GPR41. *Proceedings of the National Academy of Sciences of the United States of America* **2004**, *101*, 1045-1050, doi:10.1073/pnas.2637002100.

99. Kimura, M.; Mizukami, Y.; Miura, T.; Fujimoto, K.; Kobayashi, S.; Matsuzaki, M. Orphan G protein-coupled receptor, GPR41, induces apoptosis via a p53/Bax pathway during ischemic hypoxia and reoxygenation. *J Biol Chem* **2001**, *276*, 26453-26460, doi:10.1074/jbc.M101289200.

100. Azhar Aziz, M.; Al Mahri, S.; Al Ghamdi, A.; Mohammad, S. Role of Free Fatty Acid Receptor (FFAR3) in Growth and Proliferation of Colorectal Cancer Cell Line. *International Journal of Cancer Research* **2019**, *15*, 17-22, doi:10.3923/ijcr.2019.17.22.

101. Stoddart, L.A.; Smith, N.J.; Jenkins, L.; Brown, A.J.; Milligan, G. Conserved polar residues in transmembrane domains V, VI, and VII of free fatty acid receptor 2 and free fatty acid receptor 3 are required for the binding and function of short chain fatty acids. *J Biol Chem* **2008**, *283*, 32913-32924, doi:10.1074/jbc.M805601200.

102. Nohr, M.K.; Egerod, K.L.; Christiansen, S.H.; Gille, A.; Offermanns, S.; Schwartz, T.W.; Moller, M. Expression of the short chain fatty acid receptor GPR41/FFAR3 in autonomic and somatic sensory ganglia. *Neuroscience* **2015**, *290*, 126-137, doi:10.1016/j.neuroscience.2015.01.040.

103. Colina, C.; Puhl, H.L., 3rd; Ikeda, S.R. Selective tracking of FFAR3-expressing neurons supports receptor coupling to N-type calcium channels in mouse sympathetic neurons. *Sci Rep* **2018**, *8*, 17379, doi:10.1038/s41598-018-35690-z.

104. Inoue, D.; Kimura, I.; Wakabayashi, M.; Tsumoto, H.; Ozawa, K.; Hara, T.; Takei, Y.; Hirasawa, A.; Ishihama, Y.; Tsujimoto, G. Short-chain fatty acid receptor GPR41-mediated activation of sympathetic neurons involves synapsin 2b phosphorylation. *FEBS Lett* **2012**, *586*, 1547-1554, doi:10.1016/j.febslet.2012.04.021.

105. Won, Y.J.; Lu, V.B.; Puhl, H.L., 3rd; Ikeda, S.R. beta-Hydroxybutyrate modulates N-type calcium channels in rat sympathetic neurons by acting as an agonist for the G-protein-coupled receptor FFA3. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **2013**, *33*, 19314-19325, doi:10.1523/jneurosci.3102-13.2013.

106. Yadav, H.; Lee, J.H.; Lloyd, J.; Walter, P.; Rane, S.G. Beneficial metabolic effects of a probiotic via butyrate-induced GLP-1 hormone secretion. *J Biol Chem* **2013**, *288*, 25088-25097, doi:10.1074/jbc.M113.452516.

107. Mukherji, A.; Kobiita, A.; Ye, T.; Chambon, P. Homeostasis in intestinal epithelium is orchestrated by the circadian clock and microbiota cues transduced by TLRs. *Cell* **2013**, *153*, 812-827, doi:10.1016/j.cell.2013.04.020.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

108. Haber, A.L.; Biton, M.; Rogel, N.; Herbst, R.H.; Shekhar, K.; Smillie, C.; Burgin, G.; Delorey, T.M.; Howitt, M.R.; Katz, Y., et al. A single-cell survey of the small intestinal epithelium. *Nature* **2017**, *551*, 333-339, doi:10.1038/nature24489.

109. Nadjsombati, M.S.; McGinty, J.W.; Lyons-Cohen, M.R.; Jaffe, J.B.; DiPeso, L.; Schneider, C.; Miller, C.N.; Pollack, J.L.; Nagana Gowda, G.A.; Fontana, M.F., et al. Detection of Succinate by Intestinal Tuft Cells Triggers a Type 2 Innate Immune Circuit. *Immunity* **2018**, *49*, 33-41.e37, doi:10.1016/j.immuni.2018.06.016.

110. Schneider, C.; O'Leary, C.E.; von Moltke, J.; Liang, H.E.; Ang, Q.Y.; Turnbaugh, P.J.; Radhakrishnan, S.; Pellizzon, M.; Ma, A.; Locksley, R.M. A Metabolite-Triggered Tuft Cell-ILC2 Circuit Drives Small Intestinal Remodeling. *Cell* **2018**, *174*, 271-284.e214, doi:10.1016/j.cell.2018.05.014.

111. Han, J.H.; Kim, I.S.; Jung, S.H.; Lee, S.G.; Son, H.Y.; Myung, C.S. The effects of propionate and valerate on insulin responsiveness for glucose uptake in 3T3-L1 adipocytes and C2C12 myotubes via G protein-coupled receptor 41. *PLoS One* **2014**, *9*, e95268, doi:10.1371/journal.pone.0095268.

112. Mielenz, M.; Seybold, C.; Sauerwein, H. Effects of short-term infusion with propionate on the mRNA expression of a putative G-protein coupled receptor 41 (GPR41) in adipose tissue of goats. *Livestock Science* **2008**, *116*, 328-331, doi:10.1016/j.livsci.2008.02.001.

113. Lemor, A.; Mielenz, M.; Altmann, M.; von Borell, E.; Sauerwein, H. mRNA abundance of adiponectin and its receptors, leptin and visfatin and of G-protein coupled receptor 41 in five different fat depots from sheep. *Journal of animal physiology and animal nutrition* **2010**, *94*, e96-101, doi:10.1111/j.1439-0396.2010.00987.x.

114. Zou, J.; Chassaing, B.; Singh, V.; Pellizzon, M.; Ricci, M.; Fythe, M.D.; Kumar, M.V.; Gewirtz, A.T. Fiber-Mediated Nourishment of Gut Microbiota Protects against Diet-Induced Obesity by Restoring IL-22-Mediated Colonic Health. *Cell Host Microbe* **2018**, *23*, 41-53 e44, doi:10.1016/j.chom.2017.11.003.

115. Trompette, A.; Gollwitzer, E.S.; Pattaroni, C.; Lopez-Mejia, I.C.; Riva, E.; Pernot, J.; Ubags, N.; Fajas, L.; Nicod, L.P.; Marsland, B.J. Dietary Fiber Confers Protection against Flu by Shaping Ly6c(-) Patrolling Monocyte Hematopoiesis and CD8(+) T Cell Metabolism. *Immunity* **2018**, *48*, 992-1005 e1008, doi:10.1016/j.immuni.2018.04.022.

116. Tan, J.; McKenzie, C.; Vuillermin, P.J.; Goverse, G.; Vinuesa, C.G.; Mebius, R.E.; Macia, L.; Mackay, C.R. Dietary Fiber and Bacterial SCFA Enhance Oral Tolerance and Protect against Food Allergy through Diverse Cellular Pathways. *Cell reports* **2016**, *15*, 2809-2824, doi:10.1016/j.celrep.2016.05.047.

117. Pan, P.; Oshima, K.; Huang, Y.W.; Agle, K.A.; Drobyski, W.R.; Chen, X.; Zhang, J.; Yearsley, M.M.; Yu, J.; Wang, L.S. Loss of FFAR2 promotes colon cancer by epigenetic dysregulation of inflammation suppressors. *Int J Cancer* **2018**, *143*, 886-896, doi:10.1002/ijc.31366.

118. Jones, R.M.; Egerod, K.L.; Engelstoft, M.S.; Pedersen, M.H.; Nøhr, M.K.; Offermanns, S.; Gille, A.; Husted, A.S.; Grunddal, K.V.; Sichlau, R.M., et al. GPR41/FFAR3 and GPR43/FFAR2 as Cosensors for Short-Chain Fatty Acids in Enteroendocrine Cells vs FFAR3 in Enteric Neurons and FFAR2 in Enteric Leukocytes. *Endocrinology* **2013**, *154*, 3552-3564, doi:10.1210/en.2013-1142 %J Endocrinology.

119. Villa, S.R.; Mishra, R.K.; Zapater, J.L.; Priyadarshini, M.; Gilchrist, A.; Mancebo, H.; Schiltz, G.E.; Layden, B.T. Homology modeling of FFA2 identifies novel agonists that potentiate insulin secretion. *J Investig Med* **2017**, *65*, 1116-1124, doi:10.1136/jim-2017-000523.

120. Swaminath, G.; Jaeckel, P.; Guo, Q.; Cardozo, M.; Weiszmann, J.; Lindberg, R.; Wang, Y.; Schwandner, R.; Li, Y. Allosteric rescuing of loss-of-function FFAR2 mutations. *FEBS Lett* **2010**, *584*, 4208-4214, doi:10.1016/j.febslet.2010.09.007.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

121. Costanzi, S. Modeling G protein-coupled receptors and their interactions with ligands. *Curr Opin Struct Biol* **2013**, *23*, 185-190, doi:10.1016/j.sbi.2013.01.008.

122. Costanzi, S. Homology modeling of class a G protein-coupled receptors. *Methods Mol Biol* **2012**, *857*, 259-279, doi:10.1007/978-1-61779-588-6_11.

123. Hudson, B.D.; Tikhonova, I.G.; Pandey, S.K.; Ulven, T.; Milligan, G. Extracellular ionic locks determine variation in constitutive activity and ligand potency between species orthologs of the free fatty acid receptors FFA2 and FFA3. *J Biol Chem* **2012**, *287*, 41195-41209, doi:10.1074/jbc.M112.396259.

124. Strausberg, R.L.; Feingold, E.A.; Grouse, L.H.; Derge, J.G.; Klausner, R.D.; Collins, F.S.; Wagner, L.; Shenmen, C.M.; Schuler, G.D.; Altschul, S.F., et al. Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences. *Proceedings of the National Academy of Sciences of the United States of America* **2002**, *99*, 16899-16903, doi:10.1073/pnas.242603899.

125. Tikhonova, I.G.; Poerio, E. Free fatty acid receptors: structural models and elucidation of ligand binding interactions. *BMC Struct Biol* **2015**, *15*, 16, doi:10.1186/s12900-015-0044-2.

126. Sharma, S.; Schiller, M.R. The carboxy-terminus, a key regulator of protein function. *Crit Rev Biochem Mol Biol* **2019**, *54*, 85-102, doi:10.1080/10409238.2019.1586828.

127. Liaw, C.W.; Connolly, D.T. Sequence polymorphisms provide a common consensus sequence for GPR41 and GPR42. *DNA Cell Biol* **2009**, *28*, 555-560, doi:10.1089/dna.2009.0916.

128. Schmidt, J.; Smith, N.J.; Christiansen, E.; Tikhonova, I.G.; Grundmann, M.; Hudson, B.D.; Ward, R.J.; Drewke, C.; Milligan, G.; Kostenis, E., et al. Selective orthosteric free fatty acid receptor 2 (FFA2) agonists: identification of the structural and chemical requirements for selective activation of FFA2 versus FFA3. *J Biol Chem* **2011**, *286*, 10628-10640, doi:10.1074/jbc.M110.210872.

129. Guha, R. On exploring structure-activity relationships. *Methods Mol Biol* **2013**, *993*, 81-94, doi:10.1007/978-1-62703-342-8_6.

130. Durham, E.; Dorr, B.; Woetzel, N.; Staritzbichler, R.; Meiler, J. Solvent accessible surface area approximations for rapid and accurate protein structure prediction. *J Mol Model* **2009**, *15*, 1093-1108, doi:10.1007/s00894-009-0454-9.

131. Stoddart, L.A.; Smith, N.J.; Milligan, G. International Union of Pharmacology. LXXI. Free fatty acid receptors FFA1, -2, and -3: pharmacology and pathophysiological functions. *Pharmacological reviews* **2008**, *60*, 405-417, doi:10.1124/pr.108.00802.

132. Hudson, B.D.; Due-Hansen, M.E.; Christiansen, E.; Hansen, A.M.; Mackenzie, A.E.; Murdoch, H.; Pandey, S.K.; Ward, R.J.; Marquez, R.; Tikhonova, I.G., et al. Defining the molecular basis for the first potent and selective orthosteric agonists of the FFA2 free fatty acid receptor. *J Biol Chem* **2013**, *288*, 17296-17312, doi:10.1074/jbc.M113.455337.

133. Rowland, I.; Gibson, G.; Heinken, A.; Scott, K.; Swann, J.; Thiele, I.; Tuohy, K. Gut microbiota functions: metabolism of nutrients and other food components. *Eur J Nutr* **2018**, *57*, 1-24, doi:10.1007/s00394-017-1445-8.

134. Frost, G.; Sleeth, M.L.; Sahuri-Arisoylu, M.; Lizarbe, B.; Cerdan, S.; Brody, L.; Anastasovska, J.; Ghourab, S.; Hankir, M.; Zhang, S., et al. The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nat Commun* **2014**, *5*, 3611, doi:10.1038/ncomms4611.

135. Thirunavukkarasan, M.; Wang, C.; Rao, A.; Hind, T.; Teo, Y.R.; Siddiquee, A.A.; Goghari, M.A.I.; Kumar, A.P.; Herr, D.R. Short-chain fatty acid receptors inhibit invasive phenotypes in breast cancer cells. *PLoS One* **2017**, *12*, e0186334, doi:10.1371/journal.pone.0186334.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

136. Larraufie, P.; Martin-Gallausiaux, C.; Lapaque, N.; Dore, J.; Gribble, F.M.; Reimann, F.; Blottiere, H.M. SCFAs strongly stimulate PYY production in human enteroendocrine cells. *Sci Rep* **2018**, *8*, 74, doi:10.1038/s41598-017-18259-0.

137. Bhutia, Y.D.; Ganapathy, V. Short, but Smart: SCFAs Train T Cells in the Gut to Fight Autoimmunity in the Brain. *Immunity* **2015**, *43*, 629-631, doi:10.1016/j.immuni.2015.09.014.

138. Frost, G.; Cai, Z.; Raven, M.; Otway, D.T.; Mushtaq, R.; Johnston, J.D. Effect of short chain fatty acids on the expression of free fatty acid receptor 2 (Ffar2), Ffar3 and early-stage adipogenesis. *Nutrition & diabetes* **2014**, *4*, e128, doi:10.1038/nutd.2014.25.

139. Kobayashi, M.; Mikami, D.; Uwada, J.; Yazawa, T.; Kamiyama, K.; Kimura, H.; Taniguchi, T.; Iwano, M. A short-chain fatty acid, propionate, enhances the cytotoxic effect of cisplatin by modulating GPR41 signaling pathways in HepG2 cells. *Oncotarget* **2018**, *9*, 31342-31354, doi:10.18632/oncotarget.25809.

140. Lin, H.V.; Frassetto, A.; Kowalik, E.J., Jr.; Nawrocki, A.R.; Lu, M.M.; Kosinski, J.R.; Hubert, J.A.; Szeto, D.; Yao, X.; Forrest, G., et al. Butyrate and propionate protect against diet-induced obesity and regulate gut hormones via free fatty acid receptor 3-independent mechanisms. *PLoS One* **2012**, *7*, e35240, doi:10.1371/journal.pone.0035240.

141. Sergeev, E.; Hansen, A.H.; Pandey, S.K.; MacKenzie, A.E.; Hudson, B.D.; Ulven, T.; Milligan, G. Non-equivalence of Key Positively Charged Residues of the Free Fatty Acid 2 Receptor in the Recognition and Function of Agonist Versus Antagonist Ligands. *J Biol Chem* **2016**, *291*, 303-317, doi:10.1074/jbc.M115.687939.

142. Pizzonero, M.; Dupont, S.; Babel, M.; Beaumont, S.; Bienvenu, N.; Blanque, R.; Cherel, L.; Christophe, T.; Crescenzi, B.; De Lemos, E., et al. Discovery and optimization of an azetidine chemical series as a free fatty acid receptor 2 (FFA2) antagonist: from hit to clinic. *J Med Chem* **2014**, *57*, 10044-10057, doi:10.1021/jm5012885.

143. Namour, F.; Galien, R.; Van Kaem, T.; Van der Aa, A.; Vanhoutte, F.; Beetens, J.; Van't Klooster, G. Safety, pharmacokinetics and pharmacodynamics of GLPG0974, a potent and selective FFA2 antagonist, in healthy male subjects. *Br J Clin Pharmacol* **2016**, *82*, 139-148, doi:10.1111/bcp.12900.

144. Milligan, G.; Shimpukade, B.; Ulven, T.; Hudson, B.D. Complex Pharmacology of Free Fatty Acid Receptors. *Chem Rev* **2017**, *117*, 67-110, doi:10.1021/acs.chemrev.6b00056.

145. Wang, Y.; Jiao, X.; Kayser, F.; Liu, J.; Wang, Z.; Wanska, M.; Greenberg, J.; Weiszmann, J.; Ge, H.; Tian, H., et al. The first synthetic agonists of FFA2: Discovery and SAR of phenylacetamides as allosteric modulators. *Bioorganic & medicinal chemistry letters* **2010**, *20*, 493-498, doi:10.1016/j.bmcl.2009.11.112.

146. Hudson, B.D.; Ulven, T.; Milligan, G. The therapeutic potential of allosteric ligands for free fatty acid sensitive GPCRs. *Current topics in medicinal chemistry* **2013**, *13*, 14-25.

147. Lee, T.; Schwandner, R.; Swaminath, G.; Weiszmann, J.; Cardozo, M.; Greenberg, J.; Jaeckel, P.; Ge, H.; Wang, Y.; Jiao, X., et al. Identification and functional characterization of allosteric agonists for the G protein-coupled receptor FFA2. *Molecular pharmacology* **2008**, *74*, 1599-1609, doi:10.1124/mol.108.049536.

148. Swaminath, G.; Jaeckel, P.; Guo, Q.; Cardozo, M.; Weiszmann, J.; Lindberg, R.; Wang, Y.; Schwandner, R.; Li, Y. Mutational analysis of G-protein coupled receptor--FFA2. *Biochemical and biophysical research communications* **2011**, *405*, 122-127, doi:10.1016/j.bbrc.2010.12.139.

149. Smith, N.J.; Milligan, G. Allostery at G protein-coupled receptor homo- and heteromers: uncharted pharmacological landscapes. *Pharmacological reviews* **2010**, *62*, 701-725, doi:10.1124/pr.110.002667.

150. Schofield, Z.V.; Croker, D.; Robertson, A.A.B.; Massey, N.L.; Donovan, C.; Tee, E.; Edwards, D.; Woodruff, T.M.; Halai, R.; Hansbro, P.M., et al. Characterisation of small molecule ligands 4CMTB and

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

2CTAP as modulators of human FFA2 receptor signalling. *Sci Rep* **2018**, *8*, 17819, doi:10.1038/s41598-018-36242-1.

151. Park, B.O.; Kim, S.H.; Kong, G.Y.; Kim, D.H.; Kwon, M.S.; Lee, S.U.; Kim, M.O.; Cho, S.; Lee, S.; Lee, H.J., et al. Selective novel inverse agonists for human GPR43 augment GLP-1 secretion. *Eur J Pharmacol* **2016**, *771*, 1-9, doi:10.1016/j.ejphar.2015.12.010.
152. Smith, N.J.; Ward, R.J.; Stoddart, L.A.; Hudson, B.D.; Kostenis, E.; Ulven, T.; Morris, J.C.; Trankle, C.; Tikhonova, I.G.; Adams, D.R., et al. Extracellular loop 2 of the free fatty acid receptor 2 mediates allosterism of a phenylacetamide ago-allosteric modulator. *Molecular pharmacology* **2011**, *80*, 163-173, doi:10.1124/mol.110.070789.
153. Kobayashi, M.; Mikami, D.; Kimura, H.; Kamiyama, K.; Morikawa, Y.; Yokoi, S.; Kasuno, K.; Takahashi, N.; Taniguchi, T.; Iwano, M. Short-chain fatty acids, GPR41 and GPR43 ligands, inhibit TNF-α-induced MCP-1 expression by modulating p38 and JNK signaling pathways in human renal cortical epithelial cells. *Biochemical and biophysical research communications* **2017**, *486*, 499-505, doi:10.1016/j.bbrc.2017.03.071.
154. Said, H.; Akiba, Y.; Narimatsu, K.; Maruta, K.; Kuri, A.; Iwamoto, K.I.; Kuwahara, A.; Kaunitz, J.D. FFA3 Activation Stimulates Duodenal Bicarbonate Secretion and Prevents NSAID-Induced Enteropathy via the GLP-2 Pathway in Rats. *Dig Dis Sci* **2017**, *62*, 1944-1952, doi:10.1007/s10620-017-4600-4.
155. Arora, T.; Rudenko, O.; Egerod, K.L.; Husted, A.S.; Kovatcheva-Datchary, P.; Akrami, R.; Kristensen, M.; Schwartz, T.W.; Backhed, F. Microbial fermentation of flaxseed fibers modulates the transcriptome of GPR41-expressing enteroendocrine cells and protects mice against diet-induced obesity. *Am J Physiol Endocrinol Metab* **2019**, *316*, E453-e463, doi:10.1152/ajpendo.00391.2018.
156. De Vadder, F.; Kovatcheva-Datchary, P.; Goncalves, D.; Vinera, J.; Zitoun, C.; Duchampt, A.; Backhed, F.; Mithieux, G. Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. *Cell* **2014**, *156*, 84-96, doi:10.1016/j.cell.2013.12.016.
157. Braniste, V.; Al-Asmakh, M.; Kowal, C.; Anuar, F.; Abbaspour, A.; Toth, M.; Korecka, A.; Bakocevic, N.; Ng, L.G.; Kundu, P., et al. The gut microbiota influences blood-brain barrier permeability in mice. *Science translational medicine* **2014**, *6*, 263ra158, doi:10.1126/scitranslmed.3009759.
158. Koh, A.; De Vadder, F.; Kovatcheva-Datchary, P.; Bäckhed, F. From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. *Cell* **2016**, *165*, 1332-1345, doi:10.1016/j.cell.2016.05.041.
159. Galvao, I.; Tavares, L.P.; Correa, R.O.; Fachi, J.L.; Rocha, V.M.; Rungue, M.; Garcia, C.C.; Cassali, G.; Ferreira, C.M.; Martins, F.S., et al. The Metabolic Sensor GPR43 Receptor Plays a Role in the Control of Klebsiella pneumoniae Infection in the Lung. *Front Immunol* **2018**, *9*, 142, doi:10.3389/fimmu.2018.00142.
160. Sun, J.; Furio, L.; Mecheri, R.; van der Does, A.M.; Lundeberg, E.; Saveanu, L.; Chen, Y.; van Endert, P.; Agerberth, B.; Diana, J. Pancreatic beta-Cells Limit Autoimmune Diabetes via an Immunoregulatory Antimicrobial Peptide Expressed under the Influence of the Gut Microbiota. *Immunity* **2015**, *43*, 304-317, doi:10.1016/j.immuni.2015.07.013.
161. Hu, Y.; Le Leu, R.K.; Christophersen, C.T.; Somashekar, R.; Conlon, M.A.; Meng, X.Q.; Winter, J.M.; Woodman, R.J.; McKinnon, R.; Young, G.P. Manipulation of the gut microbiota using resistant starch is associated with protection against colitis-associated colorectal cancer in rats. *Carcinogenesis* **2016**, *37*, 366-375, doi:10.1093/carcin/bgw019.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

162. Nastasi, C.; Candela, M.; Bonefeld, C.M.; Geisler, C.; Hansen, M.; Krejsgaard, T.; Biagi, E.; Andersen, M.H.; Brigidi, P.; Odum, N., et al. The effect of short-chain fatty acids on human monocyte-derived dendritic cells. *Sci Rep* **2015**, *5*, 16148, doi:10.1038/srep16148.

163. Chun, E.; Lavoie, S.; Fonseca-Pereira, D.; Bae, S.; Michaud, M.; Hoveyda, H.R.; Fraser, G.L.; Gallini Comeau, C.A.; Glickman, J.N.; Fuller, M.H., et al. Metabolite-Sensing Receptor Ffar2 Regulates Colonic Group 3 Innate Lymphoid Cells and Gut Immunity. *Immunity* **2019**, *51*, 871-884 e876, doi:10.1016/j.immuni.2019.09.014.

164. Sivaprakasam, S.; Gurav, A.; Paschall, A.V.; Coe, G.L.; Chaudhary, K.; Cai, Y.; Kolhe, R.; Martin, P.; Browning, D.; Huang, L., et al. An essential role of Ffar2 (Gpr43) in dietary fibre-mediated promotion of healthy composition of gut microbiota and suppression of intestinal carcinogenesis. *Oncogenesis* **2016**, *5*, e238, doi:10.1038/oncsis.2016.38.

165. Wu, W.; Sun, M.; Chen, F.; Cao, A.T.; Liu, H.; Zhao, Y.; Huang, X.; Xiao, Y.; Yao, S.; Zhao, Q., et al. Microbiota metabolite short-chain fatty acid acetate promotes intestinal IgA response to microbiota which is mediated by GPR43. *Mucosal Immunol* **2017**, *10*, 946-956, doi:10.1038/mi.2016.114.

166. Pirozzi, C.; Francisco, V.; Guida, F.D.; Gomez, R.; Lago, F.; Pino, J.; Meli, R.; Gualillo, O. Butyrate Modulates Inflammation in Chondrocytes via GPR43 Receptor. *Cell Physiol Biochem* **2018**, *51*, 228-243, doi:10.1159/000495203.

167. Fujiwara, H.; Docampo, M.D.; Riwes, M.; Peltier, D.; Toubai, T.; Henig, I.; Wu, S.J.; Kim, S.; Taylor, A.; Brabbs, S., et al. Microbial metabolite sensor GPR43 controls severity of experimental GVHD. *Nat Commun* **2018**, *9*, 3674, doi:10.1038/s41467-018-06048-w.

168. Vinolo, M.A.; Ferguson, G.J.; Kulkarni, S.; Damoulakis, G.; Anderson, K.; Bohlooly, Y.M.; Stephens, L.; Hawkins, P.T.; Curi, R. SCFAs induce mouse neutrophil chemotaxis through the GPR43 receptor. *PLoS One* **2011**, *6*, e21205, doi:10.1371/journal.pone.0021205.

169. Seljeset, S.; Siehler, S. Receptor-specific regulation of ERK1/2 activation by members of the "free fatty acid receptor" family. *J Recept Signal Transduct Res* **2012**, *32*, 196-201, doi:10.3109/10799893.2012.692118.

170. Thorburn, A.N.; Macia, L.; Mackay, C.R. Diet, metabolites, and "western-lifestyle" inflammatory diseases. *Immunity* **2014**, *40*, 833-842, doi:10.1016/j.immuni.2014.05.014.

171. Wen, T.; Aronow, B.J.; Rochman, Y.; Rochman, M.; Kc, K.; Dexheimer, P.J.; Putnam, P.; Mukkada, V.; Foote, H.; Rehn, K., et al. Single-cell RNA sequencing identifies inflammatory tissue T cells in eosinophilic esophagitis. *The Journal of clinical investigation* **2019**, *129*, 2014-2028, doi:10.1172/JCI125917.

172. Li, M.; van Esch, B.C.A.M.; Henricks, P.A.J.; Garssen, J.; Folkerts, G. Time and Concentration Dependent Effects of Short Chain Fatty Acids on Lipopolysaccharide- or Tumor Necrosis Factor α-Induced Endothelial Activation. *Frontiers in pharmacology* **2018**, *9*, 233-233, doi:10.3389/fphar.2018.00233.

173. Schulthess, J.; Pandey, S.; Capitani, M.; Rue-Albrecht, K.C.; Arnold, I.; Franchini, F.; Chomka, A.; Ilott, N.E.; Johnston, D.G.W.; Pires, E., et al. The Short Chain Fatty Acid Butyrate Imprints an Antimicrobial Program in Macrophages. *Immunity* **2019**, *50*, 432-445 e437, doi:10.1016/j.immuni.2018.12.018.

174. Chang, G.; Zhang, H.; Wang, Y.; Ma, N.; Chandra, R.A.; Ye, G.; Zhuang, S.; Zhu, W.; Shen, X. Microbial community shifts elicit inflammation in the caecal mucosa via the GPR41/43 signalling pathway during subacute ruminal acidosis. *BMC Vet Res* **2019**, *15*, 298, doi:10.1186/s12917-019-2031-5.

175. Marino, E.; Richards, J.L.; McLeod, K.H.; Stanley, D.; Yap, Y.A.; Knight, J.; McKenzie, C.; Kranich, J.; Oliveira, A.C.; Rossello, F.J., et al. Gut microbial metabolites limit the frequency of autoimmune T cells and protect against type 1 diabetes. *Nature immunology* **2017**, *18*, 552-562, doi:10.1038/ni.3713.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

176. Kim, M.H.; Kang, S.G.; Park, J.H.; Yanagisawa, M.; Kim, C.H. Short-chain fatty acids activate GPR41 and GPR43 on intestinal epithelial cells to promote inflammatory responses in mice. *Gastroenterology* **2013**, *145*, 396-406.e391-310, doi:10.1053/j.gastro.2013.04.056.

177. Sanford, J.A.; O'Neill, A.M.; Zouboulis, C.C.; Gallo, R.L. Short-Chain Fatty Acids from Cutibacterium acnes Activate Both a Canonical and Epigenetic Inflammatory Response in Human Sebocytes. *J Immunol* **2019**, *202*, 1767-1776, doi:10.4049/jimmunol.1800893.

178. Zadeh-Tahmasebi, M.; Duca, F.A.; Rasmussen, B.A.; Bauer, P.V.; Cote, C.D.; Filippi, B.M.; Lam, T.K. Activation of Short and Long Chain Fatty Acid Sensing Machinery in the Ileum Lowers Glucose Production in Vivo. *J Biol Chem* **2016**, *291*, 8816-8824, doi:10.1074/jbc.M116.718460.

179. Mosher, K.I.; Wyss-Coray, T. Go with your gut: microbiota meet microglia. *Nature neuroscience* **2015**, *18*, 930-931, doi:10.1038/nn.4051.

180. Egerod, K.L.; Engelstoft, M.S.; Grunddal, K.V.; Nohr, M.K.; Secher, A.; Sakata, I.; Pedersen, J.; Windelov, J.A.; Fuchtbauer, E.M.; Olsen, J., et al. A major lineage of enteroendocrine cells coexpress CCK, secretin, GIP, GLP-1, PYY, and neurotensin but not somatostatin. *Endocrinology* **2012**, *153*, 5782-5795, doi:10.1210/en.2012-1595.

181. Kaji, I.; Karaki, S.; Tanaka, R.; Kuwahara, A. Density distribution of free fatty acid receptor 2 (FFA2)-expressing and GLP-1-producing enteroendocrine L cells in human and rat lower intestine, and increased cell numbers after ingestion of fructo-oligosaccharide. *J Mol Histol* **2011**, *42*, 27-38, doi:10.1007/s10735-010-9304-4.

182. Karaki, S.; Kuwahara, A. Propionate-induced epithelial K(+) and Cl(-)/HCO3(-) secretion and free fatty acid receptor 2 (FFA2, GPR43) expression in the guinea pig distal colon. *Pflugers Archiv : European journal of physiology* **2011**, *461*, 141-152, doi:10.1007/s00424-010-0889-y.

183. Engelstoft, M.S.; Park, W.M.; Sakata, I.; Kristensen, L.V.; Husted, A.S.; Osborne-Lawrence, S.; Piper, P.K.; Walker, A.K.; Pedersen, M.H.; Nohr, M.K., et al. Seven transmembrane G protein-coupled receptor repertoire of gastric ghrelin cells. *Mol Metab* **2013**, *2*, 376-392, doi:10.1016/j.molmet.2013.08.006.

184. Christiansen, C.B.; Gabe, M.B.N.; Svendsen, B.; Dragsted, L.O.; Rosenkilde, M.M.; Holst, J.J. The impact of short-chain fatty acids on GLP-1 and PYY secretion from the isolated perfused rat colon. *Am J Physiol Gastrointest Liver Physiol* **2018**, *315*, G53-G65, doi:10.1152/ajpgi.00346.2017.

185. Akiba, Y.; Inoue, T.; Kaji, I.; Higashiyama, M.; Narimatsu, K.; Iwamoto, K.; Watanabe, M.; Guth, P.H.; Engel, E.; Kuwahara, A., et al. Short-chain fatty acid sensing in rat duodenum. *J Physiol* **2015**, *593*, 585-599, doi:10.1113/jphysiol.2014.280792.

186. Lee, E.Y.; Zhang, X.; Miyamoto, J.; Kimura, I.; Taknaka, T.; Furusawa, K.; Jomori, T.; Fujimoto, K.; Uematsu, S.; Miki, T. Gut carbohydrate inhibits GIP secretion via a microbiota/SCFA/FFAR3 pathway. *J Endocrinol* **2018**, *239*, 267-276, doi:10.1530/JOE-18-0241.

187. Peiris, M.; Aktar, R.; Raynel, S.; Hao, Z.; Mumphrey, M.B.; Berthoud, H.R.; Blackshaw, L.A. Effects of Obesity and Gastric Bypass Surgery on Nutrient Sensors, Endocrine Cells, and Mucosal Innervation of the Mouse Colon. *Nutrients* **2018**, *10*, doi:10.3390/nu10101529.

188. Diao, H.; Jiao, A.R.; Yu, B.; He, J.; Yu, J.; Zheng, P.; Huang, Z.Q.; Luo, Y.H.; Luo, J.Q.; Mao, X.B., et al. Stimulation of intestinal growth with distal ileal infusion of short-chain fatty acid: a reevaluation in a pig model. *RSC Advances* **2017**, *7*, 30792-30806, doi:10.1039/c7ra03730a.

189. Slawinska, A.; Dunislawska, A.; Plowiec, A.; Radomska, M.; Lachmanska, J.; Siwek, M.; Tavaniello, S.; Maiorano, G. Modulation of microbial communities and mucosal gene expression in chicken intestines

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

after galactooligosaccharides delivery In Ovo. *PLoS One* **2019**, *14*, e0212318, doi:10.1371/journal.pone.0212318.

190. Lavoie, S.; Chun, E.; Bae, S.; Brennan, C.A.; Gallini Comeau, C.A.; Lang, J.K.; Michaud, M.; Hoveyda, H.R.; Fraser, G.L.; Fuller, M.H., et al. Expression of FFAR2 by Dendritic Cells Prevents Their Expression of IL27 and is Required For Maintenance of Mucosal Barrier and Immune Response Against Colorectal Tumors in Mice. *Gastroenterology* **2020**, 10.1053/j.gastro.2019.12.027, S0016-5085(0020)30007-X, doi:10.1053/j.gastro.2019.12.027.

191. Breton, J.; Ple, C.; Guerin-Deremaux, L.; Pot, B.; Lefranc-Millot, C.; Wils, D.; Foligne, B. Intrinsic immunomodulatory effects of low-digestible carbohydrates selectively extend their anti-inflammatory prebiotic potentials. *Biomed Res Int* **2015**, *2015*, 162398, doi:10.1155/2015/162398.

192. Yang, W.; Xiao, Y.; Huang, X.; Chen, F.; Sun, M.; Bilotta, A.J.; Xu, L.; Lu, Y.; Yao, S.; Zhao, Q., et al. Microbiota Metabolite Short-Chain Fatty Acids Facilitate Mucosal Adjuvant Activity of Cholera Toxin through GPR43. *J Immunol* **2019**, *203*, 282-292, doi:10.4049/jimmunol.1801068.

193. Pan, P.; Skaer, C.W.; Stirdivant, S.M.; Young, M.R.; Stoner, G.D.; Lechner, J.F.; Huang, Y.W.; Wang, L.S. Beneficial Regulation of Metabolic Profiles by Black Raspberries in Human Colorectal Cancer Patients. *Cancer prevention research (Philadelphia, Pa.)* **2015**, *8*, 743-750, doi:10.1158/1940-6207.Capr-15-0065.

194. Pan, P.; Skaer, C.W.; Wang, H.-T.; Stirdivant, S.M.; Young, M.R.; Oshima, K.; Stoner, G.D.; Lechner, J.F.; Huang, Y.-W.; Wang, L.-S. Black raspberries suppress colonic adenoma development in ApcMin/+ mice: relation to metabolite profiles. *Carcinogenesis* **2015**, *36*, 1245-1253, doi:10.1093/carcin/bgv117.

195. Hatanaka, H.; Tsukui, M.; Takada, S.; Kurashina, K.; Choi, Y.L.; Soda, M.; Yamashita, Y.; Haruta, H.; Hamada, T.; Ueno, T., et al. Identification of transforming activity of free fatty acid receptor 2 by retroviral expression screening. *Cancer science* **2010**, *101*, 54-59, doi:10.1111/j.1349-7006.2009.01348.x.

196. Han, M.; Song, P.; Huang, C.; Rezaei, A.; Farrar, S.; Brown, M.A.; Ma, X. Dietary grape seed proanthocyanidins (GSPs) improve weaned intestinal microbiota and mucosal barrier using a piglet model. *Oncotarget* **2016**, *7*, 80313-80326, doi:10.18632/oncotarget.13450.

197. Falomir-Lockhart, L.J.; Cavazzutti, G.F.; Gimenez, E.; Toscani, A.M. Fatty Acid Signaling Mechanisms in Neural Cells: Fatty Acid Receptors. *Front Cell Neurosci* **2019**, *13*, 162, doi:10.3389/fncel.2019.00162.

198. Park, J.; Wang, Q.; Wu, Q.; Mao-Draayer, Y.; Kim, C.H. Bidirectional regulatory potentials of short-chain fatty acids and their G-protein-coupled receptors in autoimmune neuroinflammation. *Sci Rep* **2019**, *9*, 8837, doi:10.1038/s41598-019-45311-y.

199. Lopez Soto, E.J.; Gambino, L.O.; Mustafa, E.R. Free fatty acid receptor 3 is a key target of short chain fatty acid. What is the impact on the sympathetic nervous system? *Channels (Austin)* **2014**, *8*, 169-171, doi:10.4161/chan.28956.

200. Kaji, I.; Akiba, Y.; Konno, K.; Watanabe, M.; Kimura, S.; Iwanaga, T.; Kuri, A.; Iwamoto, K.; Kuwahara, A.; Kaunitz, J.D. Neural FFA3 activation inversely regulates anion secretion evoked by nicotinic ACh receptor activation in rat proximal colon. *J Physiol* **2016**, *594*, 3339-3352, doi:10.1113/JP271441.

201. Kaji, I.; Akiba, Y.; Furuyama, T.; Adelson, D.W.; Iwamoto, K.; Watanabe, M.; Kuwahara, A.; Kaunitz, J.D. Free fatty acid receptor 3 activation suppresses neurogenic motility in rat proximal colon. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society* **2018**, *30*, doi:10.1111/nmo.13157.

202. Hu, J.; Kyrou, I.; Tan, B.K.; Dimitriadis, G.K.; Ramanjaneya, M.; Tripathi, G.; Patel, V.; James, S.; Kawan, M.; Chen, J., et al. Short-Chain Fatty Acid Acetate Stimulates Adipogenesis and Mitochondrial

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

Biogenesis via GPR43 in Brown Adipocytes. *Endocrinology* **2016**, *157*, 1881-1894, doi:10.1210/en.2015-1944.

203. Nakajima, A.; Nakatani, A.; Hasegawa, S.; Irie, J.; Ozawa, K.; Tsujimoto, G.; Suganami, T.; Itoh, H.; Kimura, I. The short chain fatty acid receptor GPR43 regulates inflammatory signals in adipose tissue M2-type macrophages. *PLoS One* **2017**, *12*, e0179696, doi:10.1371/journal.pone.0179696.
204. Miyamoto, J.; Ohue-Kitano, R.; Mukouyama, H.; Nishida, A.; Watanabe, K.; Igarashi, M.; Irie, J.; Tsujimoto, G.; Satoh-Asahara, N.; Itoh, H., et al. Ketone body receptor GPR43 regulates lipid metabolism under ketogenic conditions. *Proceedings of the National Academy of Sciences of the United States of America* **2019**, *116*, 23813-23821, doi:10.1073/pnas.1912573116.
205. Hu, J.; Zhou, A.; Cheung, P.C.K.; Zheng, B.; Zeng, S.; Lin, S. Expression of GPR43 in Brown Adipogenesis Is Enhanced by Rosiglitazone and Controlled by PPARgamma/RXR Heterodimerization. *PPAR Res* **2018**, *2018*, 1051074, doi:10.1155/2018/1051074.
206. Bjursell, M.; Admyre, T.; Göransson, M.; Marley, A.E.; Smith, D.M.; Oscarsson, J.; Bohlooly, Y.M. Improved glucose control and reduced body fat mass in free fatty acid receptor 2-deficient mice fed a high-fat diet. *Am J Physiol Endocrinol Metab* **2011**, *300*, E211-220, doi:10.1152/ajpendo.00229.2010.
207. Dewulf, E.M.; Ge, Q.; Bindels, L.B.; Sohet, F.M.; Cani, P.D.; Brichard, S.M.; Delzenne, N.M. Evaluation of the relationship between GPR43 and adiposity in human. *Nutrition & metabolism* **2013**, *10*, 11, doi:10.1186/1743-7075-10-11.
208. Bellahcene, M.; O'Dowd, J.F.; Wargent, E.T.; Zaibi, M.S.; Hislop, D.C.; Ngala, R.A.; Smith, D.M.; Cawthorne, M.A.; Stocker, C.J.; Arch, J.R. Male mice that lack the G-protein-coupled receptor GPR41 have low energy expenditure and increased body fat content. *Br J Nutr* **2013**, *109*, 1755-1764, doi:10.1017/S0007114512003923.
209. Regard, J.B.; Sato, I.T.; Coughlin, S.R. Anatomical profiling of G protein-coupled receptor expression. *Cell* **2008**, *135*, 561-571, doi:10.1016/j.cell.2008.08.040.
210. Daniele, G.; Eldor, R.; Merovci, A.; Clarke, G.D.; Xiong, J.; Tripathy, D.; Taranova, A.; Abdul-Ghani, M.; DeFronzo, R.A. Chronic reduction of plasma free fatty acid improves mitochondrial function and whole-body insulin sensitivity in obese and type 2 diabetic individuals. *Diabetes* **2014**, *63*, 2812-2820, doi:10.2337/db13-1130.
211. Kimura, I.; Miyamoto, J.; Ohue-Kitano, R.; Watanabe, K.; Yamada, T.; Onuki, M.; Aoki, R.; Isobe, Y.; Kashihara, D.; Inoue, D., et al. Maternal gut microbiota in pregnancy influences offspring metabolic phenotype in mice. *Science (New York, N.Y.)* **2020**, *367*, doi:10.1126/science.aaw8429.
212. Hu, S.; Kuwabara, R.; de Haan, B.J.; Smink, A.M.; de Vos, P. Acetate and Butyrate Improve beta-cell Metabolism and Mitochondrial Respiration under Oxidative Stress. *Int J Mol Sci* **2020**, *21*, doi:10.3390/ijms21041542.
213. Ximenes, H.M.; Hirata, A.E.; Rocha, M.S.; Curi, R.; Carpinelli, A.R. Propionate inhibits glucose-induced insulin secretion in isolated rat pancreatic islets. *Cell biochemistry and function* **2007**, *25*, 173-178, doi:10.1002/cbf.1297.
214. Bodis, K.; Kahl, S.; Simon, M.C.; Zhou, Z.; Sell, H.; Knebel, B.; Tura, A.; Strassburger, K.; Burkart, V.; Mussig, K., et al. Reduced expression of stearoyl-CoA desaturase-1, but not free fatty acid receptor 2 or 4 in subcutaneous adipose tissue of patients with newly diagnosed type 2 diabetes mellitus. *Nutrition & diabetes* **2018**, *8*, 49, doi:10.1038/s41387-018-0054-9.
215. Ang, Z.; Xiong, D.; Wu, M.; Ding, J.L. FFAR2-FFAR3 receptor heteromerization modulates short-chain fatty acid sensing. *Faseb j* **2018**, *32*, 289-303, doi:10.1096/fj.201700252RR.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 May 2020 doi:10.20944/preprints202005.0037.v1

Peer-reviewed version available at Biomedicines 2020, 8, 154; doi:10.3390/biomedicines8060154

216. Engelstoft, M.S.; Egerod, K.L.; Lund, M.L.; Schwartz, T.W. Enteroendocrine cell types revisited. *Current opinion in pharmacology* **2013**, *13*, 912-921, doi:10.1016/j.coph.2013.09.018.

217. Petersen, N.; Reimann, F.; Bartfeld, S.; Farin, H.F.; Ringnalda, F.C.; Vries, R.G.; van den Brink, S.; Clevers, H.; Gribble, F.M.; de Koning, E.J. Generation of L cells in mouse and human small intestine organoids. *Diabetes* **2014**, *63*, 410-420, doi:10.2337/db13-0991.

218. Bjorkman, L.; Martensson, J.; Winther, M.; Gabl, M.; Holdfeldt, A.; Uhrbom, M.; Bylund, J.; Hojgaard Hansen, A.; Pandey, S.K.; Ulven, T., et al. The Neutrophil Response Induced by an Agonist for Free Fatty Acid Receptor 2 (GPR43) Is Primed by Tumor Necrosis Factor Alpha and by Receptor Uncoupling from the Cytoskeleton but Attenuated by Tissue Recruitment. *Mol Cell Biol* **2016**, *36*, 2583-2595, doi:10.1128/MCB.00161-16.

219. Tough, I.R.; Forbes, S.; Cox, H.M. Signaling of free fatty acid receptors 2 and 3 differs in colonic mucosa following selective agonism or coagonism by luminal propionate. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society* **2018**, *30*, e13454, doi:10.1111/nmo.13454.

220. Fuller, M.; Li, X.; Fisch, R.; Bughara, M.; Wicksteed, B.; Kovatcheva-Datchary, P.; Layden, B.T. FFA2 Contribution to Gestational Glucose Tolerance Is Not Disrupted by Antibiotics. *PLoS One* **2016**, *11*, e0167837, doi:10.1371/journal.pone.0167837.

221. Fuller, M.; Priyadarshini, M.; Gibbons, S.M.; Angueira, A.R.; Brodsky, M.; Hayes, M.G.; Kovatcheva-Datchary, P.; Backhed, F.; Gilbert, J.A.; Lowe, W.L., Jr., et al. The short-chain fatty acid receptor, FFA2, contributes to gestational glucose homeostasis. *Am J Physiol Endocrinol Metab* **2015**, *309*, E840-851, doi:10.1152/ajpendo.00171.2015.

222. Ørgaard, A.; Jepsen, S.L.; Holst, J.J. Short-chain fatty acids and regulation of pancreatic endocrine secretion in mice. *Islets* **2019**, *11*, 103-111, doi:10.1080/19382014.2019.1587976.

223. Forbes, S.; Stafford, S.; Coope, G.; Heffron, H.; Real, K.; Newman, R.; Davenport, R.; Barnes, M.; Grosse, J.; Cox, H. Selective FFA2 Agonism Appears to Act via Intestinal PYY to Reduce Transit and Food Intake but Does Not Improve Glucose Tolerance in Mouse Models. *Diabetes* **2015**, *64*, 3763-3771, doi:10.2337/db15-0481.

224. Muredda, L.; Kępczyńska, M.A.; Zaibi, M.S.; Alomar, S.Y.; Trayhurn, P. IL-1β and TNFα inhibit GPR120 (FFAR4) and stimulate GPR84 (EX33) and GPR41 (FFAR3) fatty acid receptor expression in human adipocytes: implications for the anti-inflammatory action of n-3 fatty acids. *Arch Physiol Biochem* **2018**, *124*, 97-108, doi:10.1080/13813455.2017.1364774.

225. Sykaras, A.G.; Demenis, C.; Case, R.M.; McLaughlin, J.T.; Smith, C.P. Duodenal enteroendocrine I-cells contain mRNA transcripts encoding key endocannabinoid and fatty acid receptors. *PLoS One* **2012**, *7*, e42373, doi:10.1371/journal.pone.0042373.