FREE FATTY ACID RECEPTORS IN HEALTH AND DISEASE

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GRAPHICAL ABSTRACT
Regulation of systemic energy homeostasis

CLINICAL HIGHLIGHTS
This is a review of recent research on the physiological functions of free fatty acid receptors. This article also discusses how dietary fatty acids and receptors associated with metabolic and immune diseases can be utilized as therapeutic strategies as well as obstacles to be overcome in the future.
Free fatty acids (FFAs) are important energy sources for most body tissues and are classified according to their aliphatic tail length; short-chain fatty acids (SCFAs) have fewer than 6 carbon atoms, medium-chain fatty acids (MCFAs) have 6–12 carbons, and long-chain fatty acids (LCFAs) have 12 or more carbons. In addition to their function as an energy source, FFAs show critical functions such as receptor signaling, gene expression, and regulation of systemic fuel energy homeostasis under various physiological conditions (30, 106, 212). As the physiological sensors of FFAs, members of the intracellular or nuclear lipid-binding protein families, such as fatty acid binding proteins (FABPs) and peroxisome proliferator activated receptors (PPARs), are known as functional receptors that regulate many physiological and pathophysiological processes (47, 93). However, some FFA-induced physiological responses cannot be attributed to these sensors and thus other mechanisms involving plasma membrane receptors may be expected to mediate the biological processes of FFAs.

I. INTRODUCTION

Free fatty acids (FFAs) are important energy sources for most body tissues and are classified according to their aliphatic tail length; short-chain fatty acids (SCFAs) have fewer than 6 carbon atoms, medium-chain fatty acids (MCFAs) have 6–12 carbons, and long-chain fatty acids (LCFAs) have 12 or more carbons. In addition to their function as an energy source, FFAs show critical functions such as receptor signaling, gene expression, and regulation of systemic fuel energy homeostasis under various physiological conditions (30, 106, 212). As the physiological sensors of FFAs, members of the intracellular or nuclear lipid-binding protein families, such as fatty acid binding proteins (FABPs) and peroxisome proliferator activated receptors (PPARs), are known as functional receptors that regulate many physiological and pathophysiological processes (47, 93). However, some FFA-induced physiological responses cannot be attributed to these sensors and thus other mechanisms involving plasma membrane receptors may be expected to mediate the biological processes of FFAs.

G protein-coupled receptors (GPCRs) are seven transmembrane receptors that activate the heterotrimeric G protein. The human genome contains more than 800 types of GPCRs that belong to a large gene family. As these GPCRs are involved in many diseases, the receptors present a wide range of therapeutic targets for various diseases (167). To...
date, ~350 different types of GPCRs, except for olfactory receptors, have been characterized in the human genome using bioinformatics analysis based on their sequence similarities (89, 345). Approximately 200 types of these GPCRs have been characterized as already deorphanized receptors after identification of their respective endogenous ligands. However, ~150 types of orphan GPCRs remain uncharacterized (55, 62, 229) and remain unexplored as potential drug targets.

Several groups have identified a series of orphan receptors activated by FFAs and their derivatives. To date, four free fatty acid receptors (FFARs) have received considerable attention owing to their physiological importance in various biological processes (FIGURE 1 AND TABLE 1). Of the FFARs identified, FFAR1 (GPR40) and FFAR4 (GPR120) are activated by MCFA (GPR40) and LCFA (GPR40 and GPR120), while FFAR2 (GPR43) and FFAR3 (GPR41) are activated by SCFAs (147, 214). Thus each FFAR can act as an FFA sensor with selectivity for a particular FFA carbon chain length derived from food or food-derived metabolites. FFARs have been reported to have physiological functions such as facilitation of insulin and incretin hormone secretion, adipocyte differentiation, anti-inflammatory effects, neuronal responses, and taste preferences. These physiological functions of FFARs could be considered to regulate energy and immune homeostasis (30, 106, 147, 212, 214, 215, 238). Therefore, FFARs have attracted attention as potential therapeutic targets for energy metabolism disorders and immune diseases. In this review, we focus on the recent advances in our knowledge and understanding about FFARs.

### II. FFAs AS DIETARY METABOLITES

Excessive food consumption and unbalanced diet causes metabolic syndrome such as obesity, type 2 diabetes (T2D), cardiovascular disease, fatty liver, and related disorders. Obesity is currently one of the most severe health problems worldwide, especially in Western countries, owing to the increased mortality and contribution to a range of symptoms, collectively termed metabolic syndrome, which includes obesity, hypertension, hyperglycemia, dyslipidemia, and insulin resistance (140, 281). Over the past few decades, it has become increasingly clear that all macronutrients, including carbohydrates, proteins, and lipids, play an important role in regulating energy metabolism and inflammatory responses. Obesity occurs as a result of long-term imbalances in energy in-
| Signaling | Expression | Biological Functions | Reference Nos. |
|-----------|------------|----------------------|----------------|
| **GPR40/FFAR1** | | | |
| $G_{	ext{i}}$ | Pancreatic $\beta$-cells | Potentiation of glucose-stimulated insulin secretion by fatty acids | 36, 135, 151, 163, 288, 304 |
| $G_{	ext{i}}$ | Pancreatic $\alpha$-cells (?) | Stimulation of glucagon secretion (?) | 36, 41, 86, 116, 135, 169, 351 |
| $G_{	ext{a}}$ | Enteroendocrine I, K, and L cells | Stimulation of CCK, GIP, and GLP-1 secretion | 75, 187, 192 |
| Activation of ERK | Osteoblasts/clasts | Inhibition of osteoclastogenesis and stimulation of bone formation | 50, 210, 324, 358 |
| $\beta$- Arrestin recruitment | Tongue, taste buds | Regulation of taste preference for fatty acids | 45 |
| BMDCs | Induction of differentiation of M2 macrophage | | 242 |
| | | | 36 |
| **GPR41/FFAR3** | | | |
| $G_{	ext{i}}$ | Peripheral nerve | Activation of sympathetic nervous system | 157, 160 |
| $\beta$- Arrestin recruitment | Enteroendocrine L cells | PYY and GLP-1 secretion | 49, 75, 160, 181, 279, 331 |
| | Enteroendocrine K cells | Suppression of GIP secretion | 175 |
| | White adipocytes | Leptin production | 363, 370 |
| | Myeloid DCs | Dampen Th2 cell responses | 334 |
| | Thymus | Promotion of thymic $T_{\text{reg}}$ differentiation | 221 |
| | Pancreatic $\beta$-cells | Inhibition of glucose-stimulated insulin secretion | 261, 263 |
| | | Intestinal glucogenesis | 157 |
| **GPR43/FFAR2** | | | |
| $G_{	ext{i}}$ | White adipocytes | Reduction of lipolysis, reduction of fat accumulation, suppression of insulin signal | 158 |
| $G_{	ext{a}}$ | Enteroendocrine L cells | PYY and GLP-1 secretion | 331 |
| | Intestinal epithelial cells (IECs) | Production of chemokines and cytokines, NLRP activation, and IECs protection | 156, 196 |
| | Pancreatic $\beta$-cells | Enhancement of glucose-stimulated insulin secretion | 208, 262 |
| | Colonic $T_{\text{reg}}$ | Regulation of size and function of the colonic $T_{\text{reg}}$ pool | 299 |
| | M2 macrophages | TNF-$\alpha$ secretion | 222 |
| | Neutrophils | Modulation of immune cells recruitment during inflammatory responses | |
| | Eosinophils | | |
| | Mast cells | | |
| **GPR120/FFAR4** | | | |
| $G_{	ext{i}}$ | Hypothalamus | Reduction of energy efficacy and regulation of inflammation | 73 |
| $\beta$- Arrestin recruitment | Tongue, taste buds, type II taste cell | Taste perception | 45 |
| | White adipocytes | Adipocyte differentiation, glucose uptake, lipid accumulation | 132, 240 |
| | Brown adipocytes | Induction of BAT activity, FGF21 secretion | 266, 282, 301 |
| | Beige adipocytes | Promotion of the browning of white fat | 266, 282, 301 |

Continued
take and expenses, and it affects the effector pathways of various metabolites and hormones (101). Excessive food intake, insufficient exercise, and genetic susceptibility are considered risk factors for obesity.

A. FFAs

Fatty acids (FAs) are carboxylic acids with long saturated or unsaturated aliphatic chains (31, 243). Most natural FAs consist of an unbranched chain with an even number of carbon atoms from 4 to 28. FAs are commonly present in organisms as three main classes of esters: triglycerides, phospholipids, and cholesterol esters. In any of these forms, FAs are important dietary sources for animals and important structural components of cells. Saturated FAs have no carbon-carbon double bonds, whereas unsaturated FAs have one or more carbon-carbon double bonds (7, 347). Carbon-carbon double bonds enable unsaturated FAs to exist in the cis or trans isomers, with the cis isomer being the dominant configuration for most naturally occurring unsaturated FAs. The trans configuration of FAs (trans fat) is not the naturally occurring configuration, and trans fats are produced via an artificial synthesis process (348). The differences in geometry between the various types of unsaturated FAs and between saturated and unsaturated FAs play an important role in different biological processes and in the construction of biological structures such as cell membranes. When FAs in circulating plasma are not present in their ester form, these FAs are called nonesterified FAs (NEFAs) or FFAs. FFAs are always bound to a transport protein such as albumin.

B. FFA Metabolism

In animals, FAs are synthesized primarily from carbohydrates in the liver, adipose tissue, and lactating mammary glands (113, 270) (FIGURE 2). The first important step in the conversion of carbohydrates to FAs is the conversion of carbohydrates to pyruvate via glycolysis (243). This pyruvate is then decarboxylated to form acetyl-CoA in mitochondria. However, this acetyl-CoA needs to be transported to the cytosol where FA synthesis occurs. To obtain cytosolic acetyl-CoA, the citrate produced via condensation of acetyl-CoA with oxaloacetic acid is removed from the citrate cycle and transported to the cytosol through the inner mitochondrial membrane, where it is cleaved to acetyl-CoA and oxaloacetate by ATP citrate lyase. Then, oxaloacetic acid is returned to the mitochondria as malic acid (31, 83, 243). Cytosolic acetyl-CoA is carboxylated to malonyl-CoA by acetyl-CoA carboxylase, and this is the first committed step in FA synthesis. Malonyl-CoA is involved in an iterative series of reactions that lengthen the FA chain by two carbons at a time. Thus almost all natural FAs have an even number of carbon atoms. Upon synthesis completion, FFAs almost always bind to glycerol (3 FAs bind to 1 glycerol molecule) and form triglycerides, the main storage form of FAs, thus producing the main energy source in animals. Synthesized FAs are also an important component of phospholipids, which form phospholipid bilayers essential for the construction of cell membranes.

FFAs in circulating plasma are derived by lipolysis from stored triglycerides (373). As FAs are insoluble in water, they are transported by binding to plasma albumin. Thus plasma FFA levels are limited by the availability of albumin binding sites. FFAs can be taken up from circulating plasma by all mitochondria-containing cells, and they are metabolized by β-oxidation and broken down to CO₂ and water in the mitochondria to release large amounts of energy in the form of ATP produced via β-oxidation and the citric acid cycle (207); FFAs are thus utilized as an energy source in various tissues. Although cells in the central nervous system (CNS) possess mitochondria, the main energy source in the mammalian brain is glucose. FA synthesis from carbohydrates occurs in CNS cells to enable the maintenance and production of the phospholipids required for cell membranes and organelles. Simultaneously, FFAs can be transported from the plasma to...
brain through the blood-brain barrier; the transport mechanisms depend on the carbon length of FFAs (335, 343). FAs derived from ingestion of dietary fat or from the triglycerides stored in adipose tissue are distributed to cells to serve as fuel for muscle contraction and systemic metabolism.

**C. Diet and FFAs**

Dietary FAs, especially LCFAs and MCFAs, are mainly contained in triglycerides derived from animal fats and plant oils (209, 214), whereas SCFAs are the end products of fermentation of dietary fibers by gut microbes (69, 147, 230, 332) and...
also found in fermented foods (FIGURES 3 AND 4). SCFAs and MCFAs are absorbed directly into the blood via intestinal capillaries and pass through the portal vein along with other absorbed nutrients (147, 248). In contrast, LCFAs are not released directly into the intestinal capillaries (2, 88); instead, they are absorbed by the fat walls of the intestinal villi and then reassembled into triglycerides again. The triglycerides are coated with cholesterol and protein to form apolipoproteins called chylomicrons. These chylomicrons are then released from the cells into a lymphatic capillary called the lacteal, which merges into larger lymphatic vessels. It is transported through the lymphatic system and thoracic ducts to locations with thicker arteries and veins, mainly locations close to the heart. The thoracic duct releases chylomicrons into the bloodstream via the left subclavian vein, and the chylomicrons can now transport triglycerides to tissues and store or metabolize triglycerides to supply energy (2, 88).

1. LCFAs

Essential FAs, such as linoleic and \( \alpha \)-linolenic acid, are necessary for health and must be obtained from food as they cannot be synthesized de novo (143, 292, 293). \( \alpha \)-Linolenic acid (ALA; C18:3) has double bonds separated by three carbon atoms from the methyl end (omega-3 FAs), whereas linoleic acid (LA; C18:2) has double bonds separated by six carbon atoms from the methyl end (omega-6 FAs) (143, 292, 293) (FIGURE 3). These FAs are widely present in plant oils (209). However, humans lack the ability to introduce double bonds in FAs with more than 9 and 10 carbons, counting from the carboxylic acid side. ALA and LA can be converted to longer chain omega-3 FAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as well as longer chain omega-6 FAs such as arachidonic acid through a series of desaturation and elongation steps. The conversion efficiency of ALA to EPA and DHA is thought to be low in humans, and thus EPA and DHA are recommended to be obtained via fish intake. These omega-3 and omega-6 FAs are precursors of eicosanoid inflammatory mediators such as leukotrienes (LTs), prostaglandins (PGs), thromboxanes (TXs), and resolvins. Eicosanoid products, such as PGE\(_2\) and LTB\(_4\) synthesized from arachidonic acid derived from LA, are more potent mediators of thrombo-

![Diagram of Intake and Absorption of Fatty Acids](image-url)
sis and inflammation compared with that of eicosanoid products such as PGE₃ and LTB₅ synthesized from EPA derived from ALA. Thus an unbalanced omega-6/omega-3 ratio and increased intake of omega-6 polyunsaturated fatty acids (PUFAs) contribute to thrombosis and proinflammation, thereby leading to a high prevalence of atherosclerosis, obesity, and diabetes (71, 143, 166, 292–294). Indeed, regular consumption of a diet rich in omega-3 PUFAs is associated with low incidence of these diseases, as observed in the Icelandic population, Inuit natives, and Alaska’s native Americans (3, 165, 285). Omega-6 FAs are also biosynthetic precursors of endocannabinoids, which show antinociceptive, anxiolytic, and neurogenic properties. The structure of LCFAs also affects human health. Trans fat, an unsaturated FA molecule that contains a trans double bond between carbon atoms, leads to a kinked molecule. Many studies have suggested a correlation between diets high in trans fats and diseases like atherosclerosis and coronary heart disease (348).

2. MCFAs

Medium-chain triglycerides (MCTs), triglycerides consisting of MCFAs, are a unique form of dietary fat and provide several health benefits (20, 302). MCTs are primarily available as a component of butter, coconut oil, and other natural sources (21). MCTs provide ~10% fewer calories than those provided by long-chain triglycerides (LCTs). In addition, due to shorter carbon chain lengths than those of LCFAs, MCFAs are more rapidly absorbed in the intestine because of their water solubility and are more rapidly metabolized as fuel because they are transported directly to the liver (23, 244). As a result of this accelerated metabolic conversion, instead of being stored as fat, the calories contained in MCFAs are converted very efficiently into fuel for immediate use by organs and muscles. MCFAs show such energy-enhancing properties because they do not require the presence of carnitine as a carrier (unlike LCFAs) and can be transported across the double mitochondrial membrane rapidly. Therefore, MCFAs are rapidly degraded by hepatic mitochondrial β-oxidation and an excess of acetyl-CoA is
produced, resulting in the production of ketone bodies (23, 207). This response is characterized by increased energy from the consumption of MCFAs to the rapid formation of ketone bodies. Therefore, MCTs are a good choice for individuals with high-energy demands, such as the elderly to counteract the decrease in energy production due to aging, athletes to enhance athletic performance, individuals that underwent major surgery, and individuals experiencing stunted growth (19, 22, 95, 150). Recently, MCTs have also been favorably utilized as an alternative energy source to high-protein low-carbohydrate diets by athletes seeking to increase their energy levels and enhance endurance during high-intensity exercise.

### 3. SCFAs

Because humans do not possess the enzymes essential for digestion of dietary fiber, these indigestible carbohydrates escape absorption in the gastrointestinal tract, are transported through the intestines, and are fermented in the cecum and colon by anaerobic microorganisms (FIGURE 4). This fermentation by gut microbes produces various metabolites, of which SCFAs are the major group (69, 230, 275). Gut microbes produce SCFAs as the final products of their metabolic process to maintain redox equivalence in the anaerobic environment of the gut (340). SCFAs are saturated aliphatic organic acids with 1–6 carbon atoms, and the most abundant SCFAs (≥95%) are acetate (C2), propionate (C3), and butyrate (C4) that are present in a molar ratio of ~60:20:20 in colon and feces (28, 61, 112). Depending on the diet, the total SCFA concentration changes from 70–140 mM in the proximal colon to 20–70 mM in the distal colon (332). In the cecum and colon, 95% of produced SCFAs are rapidly absorbed into the colonocytes, and the remaining 5% are maintained in feces (63, 160, 277, 332). Dietary fiber intake reduces the risk of obesity, diabetes, inflammatory bowel disease, colon cancer, and cardiovascular disease (28, 42, 61, 198). For example, SCFA supplementation with a high-fat diet (HUFF) and butyrate derived from fibers improved insulin sensitivity and increased energy expenditure in a mouse model of diet-induced obesity (267).

Recent studies have also indicated that changes in the intestinal environment, particularly the composition of gut microbiota, are closely associated with metabolic disorders and immune diseases (102, 149, 153, 320). Metagenomic analysis of the gut microbiome in obese mice and humans showed that expression of genes involved in carbohydrate metabolism is predominant. Furthermore, the transplantation of gut microbiota from obese mice into germ-free mice significantly increased adiposity in the recipient mice, which was opposite to that observed after transplantation of gut microbiota from lean mice. Additionally, in European and Chinese cohort studies despite their ethnic and dietary differences, patients with T2D showed lower butyrate production and a higher proportion of Clostridiales, the non-butyrate producing bacteria (146, 265). SCFAs obtained via gut microbial metabolism also affect host immunity and inflammation, as SCFAs are involved in intestinal immune homeostasis owing to their role in regulating T cell polarization and induction via T cell differentiation to effector cells and regulatory T cells. Additionally, SCFAs have both inhibitory and acceleratory functions on neutrophils and affect immunomodulation in monocytes and macrophages. In human monocytes, SCFAs reduce the production of tumor necrosis factor (TNF)-α and monocyte chemotactic protein-1 and increase the production of PGE₂ (59). Because SCFA-rich diets show improved metabolic function in mice and humans, a direct causal relationship between fermentation of dietary fibers and SCFAs has been established (46, 90, 160). For example, butyrate, propionate, and acetate protect against diet-induced obesity and insulin resistance, while butyrate and propionate induce gut hormone secretion and decrease food intake (185). Propionate and butyrate also promote intestinal gluconeogenesis via the gut-brain axis (65). Butyrate increases FA oxidation and thermogenesis by promoting expression of peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) and phosphorylation of adenosine monophosphate-activated kinase (AMPK) in muscle and liver tissues, and PGC-1α and mitochondrial uncoupling protein-1 (UCP-1) in brown adipose tissue (BAT) (72). In contrast, increased acetate production by dysbiosis of gut microflora in rodents activated the parasympathetic nervous system, increased glucose-stimulated insulin secretion, increased ghrelin secretion, and caused hyperphagia and obesity (255). Thus oral SCFA administration may ameliorate impaired glucose metabolism and immune dysfunction in humans. In fact, clinical trials have shown that SCFA administration was effective in the treatment of Crohn’s disease, antibiotic-related diarrhea, ulcerative colitis, and obesity (28, 46, 70, 109). Thus gut microbiota play an important role in fermenting dietary fiber to release SCFAs, which are important factors of energy metabolism and immune homeostasis.

### III. GPCR FOR FFAs

FFAs are not only essential as energy sources but also function as signaling molecules that regulate various cellular processes and physiological functions, depending on their carbon chain length. Several orphan GPCRs have been identified as receptors for FFAs (FFARs). The receptors FFAR1 and FFAR4 are activated by MCFAs and LCFA, whereas FFAR3 and FFAR2 are activated by SCFAs (FIGURE 1, TABLES 2 AND 3) (66–69, 72, 97, 108, 188). GPCRs are seven transmembrane receptors constituting a large protein family of receptors that detect extracellular molecules, and then activate intracellular signal transduction pathways and ultimately cellular responses (167, 200, 271).

When GPCRs are activated by ligands, the α subunits of heterotrimeric G proteins coupled to the receptor disas-
associate from the βγ subunits, and further affect intracellular signaling protein or directly target functional proteins depending on the type of βγ subunits such as Gs, Gi/o, Gq/11, and G12/13 (200). The effector of both Gs and Gi/o pathways is adenylate cyclase, an enzyme associated with generation of cAMP. Interaction with Gα subunits of the Gs-type promotes cAMP generation, whereas interaction with the Gi/o-type inhibits cAMP generation. The effector of both Gq/11 pathways is phospholipase C (PLC). PLC activation promotes hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) into diacylglycerol and inositol 1,4,5-trisphosphate (IP3), after which activation of IP3 receptors located at the endoplasmic reticulum leads to the release of Ca2+ from the endoplasmic reticulum. The signaling pathway of Gβγ subunits is also important. The primary effectors of Gβγ are various molecules including the isoform of adenylate cyclase, ion channels such as G protein-regulated inwardly

### Table 2. Affinity of fatty acids for FFAR1 and FFAR4

| Fatty Acids | EC50 of Fatty Acid Effects on Human FFARs, μM |
|------------|---------------------------------------------|
|            | GPR40/FFAR1 | GPR120/FFAR4 |
| Saturated fatty acids |          |            |
| C6:0/caproic acid | 46 (36) |            |
| C8:0/caprylic acid | 38 (36, 135) |            |
| C10:0/capric acid | 14-43 (36, 135) |            |
| C12:0/lauric acid | 6-12 (36, 135) |            |
| C14:0/myristic acid | 8-14 (36, 135) | 30 (117) |
| C16:0/palmitic acid | 5-7 (36, 135) | 52 (117) |
| C18:0/steaeric acid | 17 (36) | 18 (117) |
| Unsaturated fatty acids |          |            |
| C16:1n-7/palmitoleic acid | 14 (35, 36) | 0.7-3 (35, 117) |
| C18:1n-9/oleic acid | 2-40 (36, 117, 135) | 31 (117) |
| Omega-3 fatty acids |          |            |
| C18:3ω-3/ω-linolenic acid | 2-13 (35, 36, 135) | 0.5 (35, 117) |
| C20:3ω-3/eicosatrienoic acid | 11 (35) | 1 (117) |
| C20:5ω-3/eicosapentaenoic acid | 2-7 (35, 36, 135) | 2-3 (35, 117) |
| C22:6ω-3/docosahexaenoic acid | 1-4 (35, 36) | 4 (117) |
| Omega-6 fatty acids |          |            |
| C18:2ω-6/linoleic acid | 2-10 (35, 36, 135) | 1 (35, 117) |
| C18:3ω-6/γ-linolenic acid | 5-9 (35, 36) | 1 (117) |
| C20:4ω-6/dihomo-γ-linolenic acid | 7 (35) | 14 (117) |
| C20:4ω-6/arachidonic acid | 2-12 (36, 135) |            |
| C22:4ω-6/docosatetraenoic acid | 13 (35) | 16 (117) |

Reference numbers are in parentheses. References, the cells, and assay systems used to determine the 50% effective concentration (EC50) values are as follows: Ref. 35, [Ca2+]i, HEK293 cells; Ref. 36, [Ca2+]i, HEK293 cells; Ref. 117, [Ca2+]i, HEK293 cells; Ref. 135, [Ca2+]i, CHO cells. FFAR, free fatty acid receptor.

### Table 3. Affinity of fatty acids for FFAR3 and FFAR2

| Fatty Acids | EC50 of Fatty Acid Effects on Human FFARs, μM |
|------------|---------------------------------------------|
|            | GPR41/FFAR3 | GPR43/FFAR2 |
| C2:0/acetic acid | >1,000 (39, 130, 174) | 35-431 (39, 130, 174) |
| C3:0/propanoic acid | 6-127 (39, 130, 174) | 14-280 (39, 130, 174) |
| C4:0/butyric acid | 42-158 (39, 130, 174) | 28-371 (39, 130, 174) |
| C5:0/valeric acid | 42-142 (39, 130, 174) | >1,000 (39, 130, 174) |
| C6:0/caproic acid | 102-134 (36, 174) |            |

Reference numbers are in parentheses. References, the cells, and assay systems used to determine the 50% effective concentration (EC50) values are as follows: Ref. 36, GTPγS, HEK293 cells; Ref. 39, GTPγS, HEK293 cells; Ref. 130, GTPγS, HEK293 cells; Ref. 174, [Ca2+]i, CHO cells, cAMP, CHO cells. FFAR, free fatty acid receptor.
rectifying K⁺ channels and Ca²⁺ channels, and phospho-
inositide-3-kinase isoforms. β-Arrestin is also a well-
known negative regulator of GPCR signaling (104, 260).
When GPCRs are activated, β-arrestins translocate to the
cell membrane and bind to the ligand-bound receptors.
β-Arrestins hinder GPCR and G protein association,
which desensitizes GPCR signaling via G proteins. β-Ar-
restins are adaptors for clathrin adaptor protein AP2 and
clathrin itself, and binding of β-arrestin to AP-2 and
clathrin induces endocytosis of GPCR. Endocytosis me-
diates a second wave of signaling within endosomes via
β-arrestin and G protein mechanisms, which is also nec-
essary for resensitization. Moreover, as a G protein-in-
dependent signaling molecule, β-arrestins interact with
several cytoplasmic proteins by functioning as scaffold
proteins to link GPCRs to intracellular signaling path-
ways, such as the mitogen-activated protein kinase
(MAPK) cascade, resulting in cell proliferation, apo-
tosis, and immune functions.

The allosteric modulators of GPCRs and GPCR oligo-
merization also play important roles in GPCR signaling and
physiological functions (87, 104a, 186). Allosteric modula-
tors interact with binding sites that are topographically dis-
tinct from the orthosteric sites recognized by endogenous
ligands. Allosteric modulators are expected to alter classical
pharmacological responses via modulating receptor confor-
mations in the presence of orthosteric ligands. Thus an al-
losteric modulator is a ligand or molecule that alters the
innate functional properties of GPCRs. Moreover, GPCR
heterodimers can lead to changes in cognate receptor sig-
aling through the physical coupling of two receptors (82,
104a). In addition to forming receptor dimers, such as ho-
o- and heterodimers, GPCR can also form oligomers that
consist of receptor trimers, tetramers, and higher order
complexes. These oligomers have properties that differ
from those of the monomers, and the function of each re-
ceptor in an oligomer depends on its tertiary structure and
quaternary structure. Additionally, each receptor acts as an
allosteric modulator of the others in complex protomers.

GPCRs can influence many physiological functions via sig-
aling pathways and self-modulation. FFAs as GPCRs are
widely expressed in various tissues and contribute to many
important physiological functions that maintain energy and
immune homeostasis. Therefore, these FFARs have been re-
garded as new drug targets for metabolic disorders and im-
une diseases such as obesity, diabetes, asthma, and colitis.

IV. FFAR1/GPR40

A. Signaling and Affinity

FFAR1 was originally reported as a receptor coupled with
G₄ protein (135, 288, 305) that activates PLC, resulting in
increased intracellular Ca²⁺ levels by IP₃ or diacylglycerol-
induced phosphorylation of protein kinase C (PKC) (FIG-
URE 1). Therefore, determination of intracellular Ca²⁺ lev-
els is employed to check for activation of FFAR1 by ligands
in many in vitro studies. Activation of extracellular signal-
regulated kinases (ERK1/2) has been confirmed as one of
the downstream signaling cascades of FFAR1-G₄ protein
signaling (135). Interestingly, some reports suggest that
FFAR1 can couple to G₄ protein, the activation of which
reduces the production of cAMP from ATP by adenylyl
cyclase inhibition (135, 286). Furthermore, FFAR1 has
been reported to be coupled with Gᵢ protein, which in-
creases cellular cAMP levels by activation of the associated
enzyme (81). Therefore, FFAR1 signaling is thought to be
more complex than expected, and further studies are essen-
tial to understand the precise mechanism and meet clinical
demands (see below).

In 2003, FFAR1 was finally reported as the receptor ac-
tivated by MCFAs and LCFAs (36, 135, 163). TABLE 2 sum-
marizes the affinity values of FFAs to FFAR1. Briefly, both
MCFAs and LCFAs can activate FFAR1 at micromolar con-
centrations. Unsaturated FAs can activate FFAR1 rather
than saturated FAs, and DHA is the most potent agonist of
FFAR1 among unsaturated FAs. FFAR1 activation by sat-
urated FAs depends on carbon chain length, and palmitic
acid (C16) is a potent ligand among saturated FAs (237).

B. Physiological Functions

Studies have demonstrated the importance of FFAR1 in
signaling mediated by FFAs or synthetic ligands in differ-
ent biological and physiological functions and have shown
FFAR1 expression in various tissues. In fact, FFAR1 was shown to be highly expressed in pancreatic
insulin-producing β-cells, intestinal L and K cells, im-
mune cells, taste buds, and the CNS (45, 111, 135).
Therefore, the biological and physiological functions of
FFAR1 signaling have been mainly demonstrated in these
tissues (FIGURE 5 AND TABLE 1).

1. Insulin and gut hormone secretion

A previous study identified the endogenous ligands of
FFAR1 and demonstrated that FFAR1 signaling on glucose-stimulated insulin secretion (GSIS) (135).
The authors demonstrated that LCFAs such as LA and
DHA promote GSIS in MIN6 cells (mice insulinoma cells)
and that the effects of LCFAs are mitigated in FFAR1-
knockdown cells by small interference RNA (siRNA).
Later, several studies confirmed the effect of FFAR1 signal-
ning stimulated by FFAs and synthetic agonists on GSIS via
in vitro and in vivo experiments. Kristinsson et al. (164)
showed that acute palmitate treatment increases GSIS in
isolated human islets and that the antagonists ANT203
partially inhibited those effects. Steneberg et al. (304)
demonstrated that the chronic effects of FFAs on GSIS
are attenuated in the FFAR1-deficient mice, whereas β-cell-specific FFAR1 overexpression prevented the development of hyperglycemia in HFD (58 kcal% fat)-induced obese mice. In addition, Nagasumi et al. (219) reported that β-cell-specific FFAR1 overexpression in KK mice, a model of obesity-associated diabetes, exhibited improvement in insulin secretion and glucose tolerance. In contrast, a later study suggested that FFAR1 contributes to the insulin secretory response, which was observed after acute FFA treatment in mice but not after chronic treatment with FFAs (171).

Transcriptional regulation of FFAR1 in pancreatic β-cells is also under investigation; however, the precise mechanism is not yet fully understood. Kebede et al. (152) indicated that glucose regulates FFAR1 expression in islets isolated from mice and humans and that it requires Pdx-1 binding to the HR2 region of the promoter. Interestingly, Natalicchio et al. (226) found that although exendin-4, a GLP-1 receptor agonist, downregulates FFAR1 expression in islets from humans and mice, and in INS-1E cells, it suppresses palmitoleate-induced apoptosis and MKK4/MKK7-dependent activation of JNK and p38 MAPK.

FFAs can stimulate hormone release from enteroendocrine cells in the intestine via FFA receptors, including FFAR1. Of the intestinal enteroendocrine cells, type L, K, and I cells express FFAR1, and these cells release specific hormones following FFA-mediated FFAR1 activation (76, 187, 252). The L and K type cells produce and secrete incretin hormones such as glucagon like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP), respectively. Edfalk et al. (76) showed that acute oral fat feeding stimulates both GLP-1 and GIP secretion in wild-type mice and that such stimulation does not occur in FFAR1-deficient mice; therefore, FFAR1-deficient mice treated with fat acutely exhibited low insulin levels and high glucose levels. In addition to K and L cells, I-type enteroendocrine cells secrete cholecystokinin (CCK) upon stimulation by FFAs; FFAR1 activation is one such mechanism of secretion. Liou et al. (187) reported that LCFA treatment increases intracellular Ca2+ levels and CCK secretion in I cells isolated from transgenic mice with CCK promoter-driven-enhanced green fluorescent protein (CCK-eGFP). In contrast, LCFA treatment did not induce CCK secretion but resulted in a slight increase in intracellular Ca2+ levels in cells isolated from FFAR1-deficient mice expressing CCK-eGFP compared with the wild type. In addition, compared with wild-type mice, FFAR1-deficient mice exhibited decreased CCK secretion in response to olive oil gavage (187). Interestingly, Hira et al. (115) indicated that FAs such as 10-oxo-trans-11-18:1, 13-oxo-cis-9, cis-15-18:2, produced from LA or ALA by gut lactic acid bacteria, stimulate CCK secretion.

**FIGURE 5.** Schematic illustration of associated biological outcomes for GPR40/FFAR1. GPR40/FFAR1 is reported to be expressed in the central nervous system, pancreatic β-cells, enteroendocrine cells, osteogenesis-related cells, and bone marrow-derived cells. GPR40/FFAR1 mediates various biological processes in each cell as illustrated. CCK, cholecystokinin; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide 1.
2. Taste

Recent studies have suggested that the taste system detects fats, carbohydrates, and proteins. FFAR1 and FFAR4 (see sect. V) are expressed in the taste buds and thus play a critical role in sensing fats. Cartoni et al. (45) reported FFAR1 expression on the back of the tongue, including the circumvallate and foliate papillae in mice. In addition, the authors indicated that FFAR1- and FFAR4-deficient mice exhibited a diminished preference to FAs during either short- or long-term two-bottle preference tests (45). However, FFAR1 expression was not confirmed in human taste buds (96). Therefore, FFAR1 may not be important for taste preference in humans.

3. Anti-inflammation

FFAR1 is reported to be involved in anti-inflammatory responses induced by FAs. Hidalgo et al. (111) showed that oleic acid increases ERK1/2 phosphorylation, superoxide production, CD11b expression, and matrix metalloproteinase-9 release in an intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) dependent manner in bovine neutrophils expressing FFAR1. In addition, the authors showed that GW9508, a FFAR1 partial agonist, induces intracellular calcium mobilization and ERK2 phosphorylation. Fujita et al. (91) suggested that FFAR1 activation by GW9508 suppresses the induction of cytokines and chemokines via proinflammatory cytokines in keratinocytes and attenuates allergic inflammation in the skin. Moreover, a recent study by Nagatake et al. (220) revealed that 17,18-epoxyeicosatetraenoic acid, a metabolite of eicosatetraenoic acid, acts as a FFAR1 ligand and exhibits antiinflammatory and anti-inflammatory effects by inhibiting neutrophil mobility in mouse and cynomolgus macaque models of contact hypersensitivity.

4. Nervous system

FFAR1 is widely expressed in neural cells, including neurons of the cerebral cortex, hippocampus, amygdala, hypothalamus, cerebellum, and spinal cord (194). Ma et al. (195) reported that DHA induces neuronal differentiation and enhances neurite growth and branching in cultured rat neuronal stem cells transfected with the FFAR1 gene. Later, Zamarbide et al. (372) showed that FFAR1 is activated by DHA in human neuroblastoma cells and that FFAR1 activation induces phosphorylation of cAMP response element-binding protein (CREB) and ERK1/2. CREB signaling is known to regulate the expression of genes that promote synaptic and neural plasticity, including the gene for brain-derived neurotrophic factor (BDNF). Furthermore, several studies reported the importance of FFAR1 in specific rodent behaviors. Nishinaka et al. (232) demonstrated that repeated intracerebroventricular injection of GW9508, the partial agonist and allosteric modulator of FFAR1 and the full agonist of FFAR4 (see sect. V; TABLE 4) (35), in mice significantly reduced the immobility time in a forced swim test, suggesting that activation of FFAR1/FFAR4 suppressed depression-like behavior. In addition, the forced swim test reduced both DHA and arachidonic acid content by ~40% in the mouse brain; these findings imply that GW9508 compensates for the potency of endogenous agonists such as DHA and arachidonic acid in the brain. Moreover, Aizawa et al. (6) revealed that FFAR1-deficient mice showed reduced sucrose preference and intake in the sucrose preference test, suggesting that FFAR1 signaling contributes to the suppression of depression-like behavior. Interestingly, the study revealed that FFAR1-deficient mice exhibit decreased anxiety-like behavior in elevated plusmaze or open-field tests concurrent with increased noradrenaline levels in the hippocampus, medulla oblongata, hypothalamus, and midbrain. Deletion of FFAR1 may lead to the development of abnormal noradrenergic neurons because FFAR1 is expressed in these neurons and can alter emotional behavior due to the abnormal noradrenaline state in the brain (6). Furthermore, previous studies demonstrated that FAs, which are endogenous ligands of FFAR1 (TABLE 2), can modulate several emotional behaviors but that the action of FFAR1 signaling may depend on ligands. For instance, rodents fed a low omega-3 FA diet showed aggressive and depressive behavior, while rodents exposed to high levels of saturated FAs showed anxiety- and aggression-like behavior as well (68). Therefore, these results suggest that FFAR1 signaling is associated with the pathology of neuropsychiatric disorders, including anxiety disorders and depression, and that the function of the signal may depend on the ligands.

5. Pain control

FFAR1 signaling possibly attenuates pain. As mentioned in the previous section, FFAR1 is expressed in the CNS, including the pain-related regions of the brain (194, 224). Nakamoto et al. (224) reported that intracerebroventricular injection of DHA or GW9508 significantly attenuated Formalin-induced pain in mice. Furthermore, the group demonstrated that inhibition of FFAR1 signaling by repeated administration of the FFAR1 antagonist GW1100 exacerbated mechanical allodynia in mice (223). Incision-induced mechanical allodynia was exacerbated in FFAR1-deficient mice compared with wild-type mice, although responses in the plantar test did not change (223). It has been suggested that the midbrain area, including the periaqueductal gray or locus coeruleus, and hypothalamus are associated with pain modulation (194, 225). In addition, several FFAs including DHA were found increased in the hypothalamus and midbrain of mice during the early phase of pain inflicted by plantar incision (postoperative pain mouse model) (223, 259). A
### Table 4. Summary of synthetic ligands for FFARs

| Ligands                  | Action                                      | Half-Maximal Activities, \( \mu M \) | Therapeutic Target                                      | Reference Nos. |
|--------------------------|---------------------------------------------|----------------------------------------|--------------------------------------------------------|----------------|
| **GPR40/FFAR1**          |                                             |                                        |                                                        |                |
| Rosiglitazone            | Full agonist                                | 2.8<sup>a</sup>                       | Type 2 diabetes                                        | 163            |
| MEDICA 16                | Full agonist                                | 1.22<sup>a</sup>                      | Type 2 diabetes                                        | 163            |
| GW9508                   | Partial agonist                             | 0.048<sup>b</sup>                     | Type 2 diabetes                                        | 35             |
| GWV1 100                 | Antagonist                                  | 1<sup>b</sup>                         | Type 2 diabetes                                        | 35             |
| AMG-837                  | Allosteric partial agonist                  | 0.0015–0.12<sup>c</sup>               | Type 2 diabetes                                        | 184, 185, 192  |
| TUG424                   | Partial agonist                             | 0.032<sup>d</sup>                     | Type 2 diabetes                                        | 50, 52, 289    |
| TUG-770                  | Agonist                                     | 0.006<sup>b</sup>                     | Type 2 diabetes                                        | 49             |
| DS-1558                  | Agonist                                     | 0.0038<sup>c</sup>                    | Type 2 diabetes                                        | 317            |
| Compound 40              | Full agonist                                | 0.02<sup>b</sup>                      | Type 2 diabetes                                        | 49             |
| NCG21                    | Agonist                                     | 20<sup>b</sup>                        | Type 2 diabetes                                        | 311, 313       |
| Compound 43              | Agonist                                     | 65<sup>g</sup>                        | Type 2 diabetes                                        | 289            |
| TUG891                   | Orthosteric agonist                         | 5.011<sup>b</sup>, 0.064<sup>g</sup> | Metabolic and inflammatory processes, type 2 diabetes  | 127, 128, 289  |
| TAK-875                  | Agonist                                     | 0.014<sup>c</sup>                     | Type 2 diabetes                                        | 228            |
| Compound 1               | Partial agonist (for G<sub>i</sub>)          | 0.072<sup>b</sup>, 0.1258<sup>i</sup> | Type 2 diabetes                                        | 118            |
| Compound 9               | Agonist                                     | 0.38<sup>b</sup>                      | Type 2 diabetes                                        | 138            |
| **GPR41/FFAR3**          |                                             |                                        |                                                        |                |
| AR420626                 | Agonist                                     | 0.27<sup>i</sup>, 0.117<sup>i</sup>  | Type 2 diabetes, GLP-1 secretion, ghrelin secretion    | 78, 234        |
| Compound 3               | Positive allosteric modular agonist         | 3.47<sup>h</sup>                      | Inflammatory processes                                 | 124            |
| **GPR43/FFAR2**          |                                             |                                        |                                                        |                |
| Compound 1               | Orthosteric agonist                         | 0.072<sup>b</sup>                     | Metabolic and inflammatory conditions                  | 126            |
| 4-CMTB                   | Ago-allosteric modulator                    | 6.38<sup>h</sup>                      | Inflammation                                           | 298            |
| CFMB                     | Allosteric agonist                          | 0.7<sup>i</sup>                       | Immune and inflammatory responses                      | 178            |
| Compound 58/PA           | Allosteric modulator                        | 0.7<sup>mm</sup>                      | Obesity, insulin resistance, and diabetes              | 208, 352       |
| SCA14                    | Orthosteric agonist                         | 3.2<sup>e</sup>                       | Hyperglycemia and type 2 diabetes                      | 262, 284       |
| SCA15                    | Orthosteric agonist                         | 2.7<sup>e</sup>                       | Hyperglycemia and type 2 diabetes                      | 262, 284       |
| AMG-7703                 | Allosteric agonist                          | 0.45<sup>c</sup>                      | Inflammation and metabolic disorders                  | 178, 315, 352  |
| Euroscreen compound series | Orthosteric agonist                        | 0.013–0.06<sup>e</sup>               | Inflammatory, gastrointestinal, and/or metabolic disorders | 212, 237  |
| Euroscreen compound series | Antagonist                                | 0.006–0.02<sup>e</sup>               | Metabolic disorders and inflammatory diseases        | 212, 237       |
| Galapagos compounds      | Antagonist                                  | <0.01<sup>c</sup>                     | Metabolic disorders and inflammatory diseases        | 212, 237       |
| **GPR120/FFAR4**         |                                             |                                        |                                                        |                |
| GW9508                   | Full agonist                                | 3.5<sup>b</sup>                       | Type 2 diabetes                                        | 35             |
| GSK137647A               | Full agonist                                | 0.5<sup>i</sup>                       | Mitigating excess food intake and limiting obesity risk | 201            |
| Compound 40              | Full agonist                                | 4.8<sup>b</sup>                       | Type 2 diabetes                                        | 49             |
| NCG21                    | Agonist                                     | 1.3<sup>b</sup>                       | Type 2 diabetes                                        | 311, 313       |
| Compound 43              | Agonist                                     | 0.044<sup>g</sup>                     | Metabolic and inflammatory processes, type 2 diabetes  | 289            |

*Continued*
and obesity, and several have been tested in clinical trials; however, none of the drugs has yet been approved for clinical use. FFAR1 activation was shown to promote insulin secretion and glucose tolerance, the development of synthetic FFAR1 agonists has been explored as a therapeutic target for T2D and obesity. Numerous compounds targeting FFAR1 were discovered and suggested as drugs for T2D (363), with TAK-875 exhibiting positive cooperativity with the endogenous FFAR1 ligand GLP-1. It has also been reported as a potent, selective, and orally bioavailable FFAR1 agonist (228). Tsujihata et al. (336) demonstrated that TAK-875 enhances GSIS with no effects on glucagon secretion and improves hyperglycemia in either fasting or postprandial conditions in the diabetic model rats. Yashiro et al. (366) also showed that TAK-875 increases GSIS in a glucose-dependent manner in isolated rat and human pancreatic islets. Later, Yabuki et al. (364) reported that TAK-875 stimulates Ca\textsuperscript{2+} in human HEK293 cells overexpressing FFAR1 (EC\textsubscript{50} = 19 nM) (35, 251). It has also been reported as a partial agonist and allosteric modulator that ameliorates the potency of PUFA to FFAR1 (35). Despite being a full agonist of FFAR4, GW9508 activates more FFAR1 than FFAR4 (TABLE 4) (35). TAK-875 was also reported as a potent, selective, and orally bioavailable FFAR1 agonist (228). Tsujihata et al. (336) demonstrated that TAK-875 enhances GSIS with no effects on glucagon secretion and improves hyperglycemia in either fasting or postprandial conditions in the diabetic model rats. Yashiro et al. (366) also showed that TAK-875 increases GSIS in a glucose-dependent manner in isolated rat and human pancreatic islets. Later, Yabuki et al. (364) reported that TAK-875 is an ago-allosteric modulator of FFAR1 because TAK-875 exhibits positive cooperativity with the endogenous ligands, such as γ-LA, in cell lines and mouse islets. Although a phase III clinical trial was performed to test this molecule as a potential therapeutic agent for T2D, the molecule was withdrawn from the trial owing to liver toxicity (141). P11187 is a potent oral FFAR1 antagonist, and phase I clinical trials have been completed; however, no further information is available for this compound. JTT-851, an orally active FFAR1 agonist, underwent phase II clinical trials; however, the development of this compound has been discontinued (182). TUG-770, another potent FFAR1 agonist that has favorable physicochemical and pharmacokinetic properties, has been shown to improve glucose tolerance in diet-induced obese mice (51). Moreover, DS-1558 was found as a potent and orally active FFAR1 agonist, which was shown to improve insulin secretion and glucose homeostasis in Zucker diabetic fatty rats (317). AM-1638 and AM-5262 were discovered as full allosteric FFAR1 agonists that activate both Gq and Gs proteins (40, 110, 192, 256, 353). These AM compounds directly stimulate insulin secretion and promote incretin release from enteroendocrine cells, and the effects of AM-5262 appear to be stronger than those of AM-1638. In addition, AP1 and AP3, full agonists of FFAR1, were reported to stimulate insulin and incretin in diabetic GK (Goto-Kakizaki) rats and reduce body weight and blood glucose levels (246).
(107) indicated that FFAR4 activation by agonists such as synthetic ligands and LCFAs increases intracellular Ca\(^{2+}\) levels without cAMP production in human or mouse FFAR4-expressing cells, suggesting that FFAR4 is coupled with the G\(_{q}\) protein but not the G\(_{i/o}\) or G\(_{s}\) proteins. As for downstream signaling, activation of ERK1/2 and phosphoinositide 3-kinase were confirmed following the activation of FFAR4 under the specific study conditions; however, the precise cascade remains unclear (148).

In 2005, Hirasawa et al. (117) deorphanized FFAR4 as the second FFAR and showed that it has strong affinity to LCFAs. The report described that GLP-1 secretion is stimulated by FFAs via FFAR4 activation in STC-1 cells and that plasma insulin and GLP-1 levels were increased in the mice orally treated with ALA. Although FFAR1 and FFAR4 share only 10\% homology between their amino acid sequences (117, 135), ligand activity of FAs to FFAR4 is similar to that to FFAR1. FFAR4 prefers C18 PUFAs as endogenous ligands, per the 50\% effective concentration (EC\(_{50}\)) values obtained using cells overexpressing these GPR proteins (TABLE 2) (131).

### B. Physiological Functions

Although FFAR4 is widely expressed in different tissues and cell types, higher FFAR4 expression was observed in the intestine, immune cells, taste buds, and adipocytes (106). Consistent with the expression pattern, FFAR4 is implicated in various biological and physiological functions such as energy regulation, immunological homeostasis, and neuronal functions (FIGURE 6 AND TABLE 1).

#### 1. Gut hormone secretion

The deorphanization study of FFAR4 also indicated that FFAR4 stimulation by FFAs promotes the secretion of GLP-1 in vitro or in vivo and that it increases circulating insulin levels in mice (117). Consistent with this, FFAR4

![Figure 6](image-url)

**FIGURE 6.** Schematic illustration of associated biological outcomes for GPR120/FFAR4. GPR120/FFAR4 is reported to be expressed in the hypothalamus, taste buds, white adipocytes, brown and beige adipocytes, enteroendocrine cells, pancreatic delta-cells, macrophages (M\(_f\)), and osteogenesis-related cells. GPR120/FFAR4 mediates various biological processes in each cell as illustrated. BAT, brown adipose tissue; CCK, cholecystokinin; FGF21, fibroblast growth factor-21; GLP-1, glucagon-like peptide 1.
expression was confirmed in GLP-1-expressing enteroendocrine cells in the mouse colon (117). Other studies also confirmed that FFAs promote GLP-1 and CCK secretion from enteroendocrine cell lines, such as STC-1, that endogenously express FFAR4 (107, 291), whereas the effects were reduced in cells transfected with FFAR4-specific shRNA (321). Furthermore, Sundstrom et al. (312) indicated that FFAR4 activation by the agonist AZ13581837 resulted in low plasma glucose levels and increased insulin and GLP-1 secretion after intravenous glucose administration in wild-type mice but not in FFAR4-deficient mice. In addition, a GLP-1 receptor antagonist, exendin 9–39, eliminated the effects of FFAR4 activation on plasma glucose and insulin secretion in wild-type mice (312). Furthermore, Iwasaki et al. (136) revealed the expression of FFAR4 in enteroendocrine K cells, which are widely present in the upper small intestine and are known to secrete GIP. The authors demonstrated that lard oil feeding stimulated GIP secretion and decreased plasma glucose levels in wild-type mice but not in FFAR4-deficient mice as evidenced by oral glucose tolerance tests. In contrast, Paulsen et al. (253) did not observe any increase in circulating GLP-1 levels following administration of ALA, a FFAR4 ligand, in rats. Numerous in vivo and in vitro studies support the notion that FFAR4 activation is associated with the secretion of incretin hormones, including GLP-1 and GIP; however, further studies are warranted to understand the underlying mechanisms.

2. Anti-inflammation and insulin sensitivity

FFAR4 is highly expressed in adipose tissue and proinflammatory macrophages, indicating that FFAR4 plays important roles in these tissues and cell types. Oh et al. (240) demonstrated the anti-inflammatory functions of FFAR4 in the presence of ligands DHA and EPA in monocytic RAW264.7 cells and in primary intraperitoneal macrophages. Using an in vitro system, the authors also showed that the mechanism underlying the DHA-mediated anti-inflammatory response, such as inhibition of both the TLR and TNF-α signaling pathways, via FFAR4 involves inhibition of TAK1 phosphorylation via a β-arrestin-2/TAK1 binding protein 1 (TAB1)-dependent effect. In addition, in vivo experiments revealed that omega-3 FA treatment leads to the amelioration of HFD (60 kcal% fat)-induced tissue inflammation, thereby improving systemic insulin sensitivity in wild-type mice; these effects were not observed in FFAR4-deficient mice (239). Yamada et al. (365) indicated that eicosatetraenoic acid attenuates the palmitate-induced increase in inflammatory gene expression and NF-κB phosphorylation in 3T3-L1 adipocytes, but that silencing of FFAR4 via siRNA suppresses the anti-inflammatory effects of EPA. Moreover, they demonstrated that EPA supplementation attenuates inflammatory signal transduction and macrophage phenotype in adipose tissues of mice fed a high-fat, high-sucrose diet; however, they did not confirm whether this EPA-induced effect occurred via FFAR4. Conversely, Pørregaard et al. (247) suggested that the effect of omega-3 FA on inflammation and insulin resistance is independent of FFAR4 signaling based on a comparison with the results of omega-6 FA treatment in FFAR4-deficient mice.

Xiao et al. (375) reported the expression of FFAR4 in human and rat pancreas and that FFAR4 colocalizes with CD68 (specific marker of macrophages) as well as CD34 and CD117 (markers of interstitial cells in the pancreas) via immunohistological analysis. Stone et al. (307) further demonstrated the colocalization of FFAR4 with somatostatin in FFAR4-deficient/β-galactosidase knock-in mice, suggesting that FFAR4 is selectively expressed in islet delta cells.

Furthermore, Konno et al. (162) characterized the expression and function of FFAR4 in human eosinophils via in vitro experiments using synthetic agonists and revealed that stimulation of FFAR4 by a synthetic ligand suppresses spontaneous apoptosis and increases the release of interleukin (IL)-4. These results indicate that FAs through FFAR4 may facilitate local immune responses in tissues such as adipose tissues and the intestine, which are the usual sites of eosinophil localization.

FFAR4 may also partially exhibit anti-inflammatory roles in intestinal epithelial cells. Anbazhagan et al. (9) demonstrated that treatment of Caco-2 cells with FFAR4 agonists, such as GW9508, TUG-891, or DHA, increases β-arrestin-2-TAB1 interaction, attenuates TAB1 binding to TAK1, and inhibits NF-κB activation. In addition, a recent report showed that the branched palmitic acid esters of hydroxy stearic acids act as endogenous ligands of FFAR4 and that the branched FA esters of hydroxy FAs protect against experimental colitis in the gut of mice treated with dextran sodium sulfate by altering the innate and adaptive immune responses (176). These studies support the use of FFAR4 agonists in the prevention and treatment of intestinal inflammatory diseases; however, further confirmatory studies are required.

FFAR4 has been reported to be expressed in mature osteoclasts and osteoblasts, indicating regulation of bone metabolism by FFAR4 signaling; however, FFAR4 deletion did not result in changes in the bone mass of mice (5, 56, 155). Interestingly, Ahn et al. (5) reported that bone loss occurs in HFD (60 kcal% fat)-induced obese mice and that FFAR4 signaling promotes osteoblastic bone formation in HFD-fed Fat-1 transgenic mice that are capable of producing omega-3 FAs from omega-6 FAs. These findings suggest that increased endogenous omega-3 FAs prevent bone loss via FFAR4 signaling in osteoclasts and osteoblasts under obese conditions. As another possibility, bone loss may be inhibited by the secretion of adipocyte-derived hormones that
affect bone development because FFAR4 is important for adipocyte differentiation (see sect. VB3).

3. Adipogenesis and lipid accumulation

FFAR4 is widely expressed in adipocytes and adipose tissue but has not been detected in preadipocytes (240, 365). Ichimura et al. (132) demonstrated that HFD (60 kcal% fat)-fed FFAR4-deficient mice exhibit an obesity phenotype with decreased adipocyte differentiation and lipogenesis compared with that of HFD (60 kcal% fat)-fed wild-type mice, suggesting a key role for FFAR4 in lipid sensing and energy control. Gotoh et al. (100) showed that FFAR4, but not FFAR1, is highly expressed in white adipose tissues (subcutaneous, perirenal, mesenteric, and epididymal) in mice and that its expression levels are elevated in adipose tissues of HFD (60 kcal% fat)-fed mice compared with control diet-fed mice. In addition, the report indicated that FFAR4 expression is increased in 3T3-L1 cells during adipogenic differentiation, when intracellular lipids are accumulated (100); siRNA-mediated downregulation of FFAR4 expression inhibited adipocyte differentiation and lipid accumulation in 3T3-L1 cells. Furthermore, Liu et al. (189) also silenced FFAR4 via siRNA and reported decreased lipid accumulation in 3T3-L1 cells and a significant reduction in gene and protein levels of IRS-1 and GLUT4, suggesting a contribution of FFAR4 to glucose metabolism and insulin resistance.

A recent report by Quesada-López et al. (266) indicated that FFAR4 activation promotes BAT activity and the browning of white fat in mice via the hormonal factor fibroblast growth factor-21, suggesting that thermogenic regulation is mediated by FFAR4; FFAR4-deficient mice showed impaired cold-induced browning.

4. Taste

Compared with FFAR1, FFAR4 is a more important element of the taste response system. FFAR4 mRNA is significantly expressed in the epithelium of the circumvallate papillae but not in the nonsensory epithelium of rodents (45, 205, 206). Cartoni et al. (45) showed that FFAR4-deficient mice exhibited a diminished preference to FAs in either 48-h two-bottle preference tests or brief access tests; this indicates that thermogenic regulation is mediated by FFAR4; FFAR4-deficient mice showed impaired cold-induced browning.

5. Nervous system

Despite the fact that FFAR4 expression has been detected in the CNS, its role in the CNS remains poorly understood. The hypothalamic inflammatory response caused by excessive energy intake is linked to the induction of obesity and T2D (64, 211). Auguste et al. (17) demonstrated that intracerebroventricular injection of the FFAR4 agonist GPR120 III reduces food intake and suppresses the rewarding effects of a high-fat, high-sucrose diet in mice. Accordingly, FFAR4 signaling may improve feeding behavior and energy homeostasis by attenuating neuroinflammation, although peripheral FFAR4 is known to control these functions via regulation of gut hormone secretion (see sect. VB1). Furthermore, several studies have confirmed the importance of omega-3 FAs, such as DHA and EPA, in CNS functions including mood stability and emotional control (68, 308, 355), which suggests a potential role for FFAR4 signaling in emotional behavior. In addition, Wellhauser et al. (359) reported that activation of FFAR4 by DHA is sufficient for decreasing the inflammatory response upon TNF-α exposure at the transcriptional and translational level in rHypoE-7 cells, a hypothalamic neuron model. Further investigations are needed to elucidate the importance of FFAR4 signaling in the nervous system.

C. Pharmacology

Ichimura et al. (114) demonstrated that compared with HFD (60 kcal% fat)-fed wild-type mice, HFD (60 kcal% fat)-fed FFAR4-deficient mice exhibited severe obesity phenotypes, including increased body weight, fatty liver, lipogenesis, and adipocyte inflammation. In addition, the group demonstrated that FFAR4 expression in adipose tissue is significantly higher (60 kcal% fat)-fed wild-type mice, HFD (60 kcal% fat)-fed FFAR4-deficient mice exhibit an obesity phenotype. Ichimura et al. (132) demonstrated that FFAR4 rather than FFAR1 is a lipid sensor candidate.

As several studies have indicated that FFAR4 plays an important role in insulin sensitivity, synthetic ligands of FFAR4, such as TUG-891 and GW9508, have been indi-
V. GPR43/FFAR2

A. Signaling and Affinity

In studies using [Ca^{2+}], assays for screening bioactive compounds as ligands, FFAR2 was reported to be activated by acetate (C2) and other SCFAs such as propionate (C3) and butyrate (C4) (39, 231). Previous structure-activity relationship studies have shown that FFAR2 exhibits a preference for shorter SCFAs. SCFAs are primarily natural agonists of FFAR2 and show species-dependent variations in ligand response (125, 130, 349). The potencies of individual SCFAs in activating FFAR2 in humans are determined by EC_{50} values and are ordered as C2 = C3 < C4 > other SCFAs (39, 130, 174) (TABLE 3), whereas those for the mouse receptor are ordered as C2 < C3 < C4 (130). These differences between the response of human and mouse receptors to SCFAs may be because the mouse receptor shares ~84% amino acid sequence similarity with the human receptor (130). Previous studies have also demonstrated that FFAR2 is coupled with both pertussis toxin-sensitive G_{i/o} and G_{q} proteins (FIGURE 1) (11, 174). FFAR2 activation by SCFAs via the G_{i/o} family of G proteins inhibits cAMP production and activates the ERK cascade, whereas FFAR2 activation with SCFAs via the G_{q} family of G proteins results in elevation of [Ca^{2+}], and promotes activation of the MAPK cascade. Moreover, the recruitment of β-arrestin-2 induced by agonist stimulation was confirmed via a bioluminescence resonance energy transfer (BRET)-based approach in HEK293 cells cotransfected with eYFP-tagged FFAR2 and β-arrestin-2-Renilla luciferase (125, 126). FFAR2 signaling inhibited nuclear translocation of NF-κB, thereby reducing the expression of inflammatory cytokines such as IL-1β and IL-6 in FFAR2-transfected HeLa cells. This effect was eliminated when β-arrestin-2 was knocked down using siRNA (177). Both SCFA receptors, FFAR2 and FFAR3, couple with the pertussis toxin-sensitive G_{i/o} family of proteins, but only FFAR2 also couples to the pertussis toxin-insensitive G_{q} family of proteins (11, 33, 129, 174). However, the physiological significance of this dual-coupled signaling mechanism through FFAR2 remains unclear.

Mutational analysis revealed previously unidentified sites that may allosterically regulate the function of orthosteric ligands and led to the identification of residues potentially important for the interactions between orthosteric and allosteric binding sites. Several charged residues located in transmembrane domains (TM) 5, 6, and 7 are important for anchoring carboxylate groups of LCFAs in FFAR1 (309, 330). These polar residues are also conserved in FFAR2 and FFAR3 and have been shown to be important for FFAR2 and FFAR3 endogenous ligand binding and function, similar to that in FFAR1 (306, 314). The mode-1 binding motif is not a dual-coupled signaling mechanism through FFAR2 remains unclear.

Collectively, these findings support FFAR4 agonists as insulin-sensitizing agents in the treatment of metabolic disorders such as obesity and diabetes; however, further studies are essential for the successful development of FFAR4 agonist-based drugs.
B. Physiological Functions

1. Gut hormone secretion

In the intestines, FFAR2 is involved in the regulation of appetite and insulin signaling. The intestine has crucial roles in energy homeostasis, such as the secretion of incretin hormones and absorption of nutrients (24, 48, 337). Karaki et al. (144) performed immunohistochemistry analysis using an anti-FFAR2 antibody and determined that FFAR2-expressing cells are colocalized with peptide YY (PYY)-containing enteroendocrine L cells of the rat gastrointestinal tract. Enteroendocrine L cells are also one of the major cell types that express the proglucagon gene \textit{Gcg}. GLP-1 is co-stored and cosecreted with PYY by enteroendocrine L cells (154). SCFA treatment stimulated GLP-1 secretion in mixed colonic cell cultures via FFAR2 in vitro and in vivo (269, 331). Moreover, FFAR2- and FFAR3-deficient mice showed reduced SCFA-induced GLP-1 secretion and impaired glucose tolerance. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) showed that FFAR2 and FFAR3 were abundantly expressed in GLP-1-secreting L cells. Moreover, SCFAs led to increased [Ca$^{2+}$]$_i$ levels via the G$_q$ signaling pathway in L cells in primary culture. It was originally believed that nutrient and metabolite sensors are expressed and function mainly at the apical pole of enteroendocrine cells to directly sense luminal content (331). However, FFAR2 has been shown to be expressed on the basolateral membrane and along the basal extensions of enteroendocrine cells (77, 144). In contrast, Park et al. (249) demonstrated that

2. Adipogenesis and lipid accumulation

FFAR2 is expressed in adipocytes, where they regulate adipogenesis and disease pathogenesis (12, 369), although there are conflicting reports in the literature as to the exact mechanisms underlying these effects (276, 316). Recent studies have demonstrated that FFAR2 is involved in the regulation of energy accumulation in adipose tissues and contributes to the development of obesity. Hong et al. (119) attempted to elucidate the role of FFAR2 in adipocytes and showed that FFAR2 expression in white adipose tissues of mice with HFD (41 kcal% fat)-induced obesity was significantly higher than in normal chow-fed mice. In 3T3-L1 cells, FFAR2 and PPAR-\(\gamma\)-2 mRNA expression levels increased following SCFA treatment, whereas siRNA-mediated
suppression of FFAR2 mRNA inhibited adipogenesis. Moreover, SCFA treatment suppressed isoproterenol-induced lipolysis in a dose-dependent manner in 3T3-L1 cells (119). Ge et al. (98) demonstrated that these effects are mediated by FFAR2 by showing acetate-induced suppression of lipolysis and the release of glycerol by adipocytes isolated from wild-type mice in vitro. Furthermore, although FFAR2 activation via intraperitoneal injection of sodium acetate led to a rapid reduction in plasma FA levels in vivo, this effect was eliminated in FFAR2-deficient mice (98).

SCFAs are produced by microbial fermentation of dietary fiber in the gut. Kimura et al. (158) performed several in vitro and in vivo experiments and demonstrated that obesity was induced in FFAR2-deficient mice fed an HFD (60 kcal% fat), whereas mice overexpressing FFAR2 in adipose tissue were lean under normal conditions. However, germ-free conditions or antibiotic treatment eliminated these phenotypes in both mouse strains. Furthermore, FFAR2 activation by SCFAs decreased insulin signaling and inhibited fat accumulation in adipose tissue (158). Unincorporated glucose and lipids were primarily utilized by muscle tissue. Thus the FFAR2-insulin pathway may play a key role in adipose tissue by serving as a physiological mechanism for the regulation of whole-body energy balance by metabolic fuels. These findings indicate that FFAR2 is potentially relevant in the treatment of obesity and T2D. In contrast, Zou et al. (376) reported that FFAR2 deficiency did not alter the insulin-based amelioration of HFD (60 kcal% fat)-induced dysglycemia or adiposity. However, this latter result is an apparent contradiction with the results of other recent studies (37, 158), which showed HFD-induced weight gain and adiposity in wild-type mice but not in FFAR2-deficient mice. Zou et al. (376) speculate that although a portion of insulin’s beneficial metabolic effects may be mediated by SCFAs acting via FFAR2, SCFA-independent effects may mask the need for this receptor. Bjursell et al. (29) reported that HFD (39.9 kcal% fat)-fed FFAR2-deficient mice exhibited increased insulin sensitivity in old age owing to increased energy expenditure, which resulted in increased body temperature. This observation was explained by their histological analysis of BAT, which plays a critical role in the regulation of energy balance and homeostasis. Compared with HFD-fed wild-type mice, HFD-fed FFAR2-deficient mice showed decreased lipid dispersion. In addition, Lemor et al. (179) reported that FFAR2 mRNA expression in adipose tissues is higher during early lactation in dairy cattle, indicating that receptor expression is associated with the metabolic state of animals. Although there are conflicting reports that describe the biological function of FFAR2 in adipose tissue, how these findings fit within the modulation of fat metabolism remains to be defined. Adipose FFAR2 may exert physiological effects depending on the energy conditions and nutritional status of the body, and may thereby contribute to the maintenance of energy homeostasis.

3. Anti-inflammation

Initial studies have indicated that FFAR2 is expressed extensively in immune tissues such as the spleen and immune cells such as neutrophils (39, 204, 231), suggesting that SCFAs play an important role in immune responses. FFAR2 expression is also upregulated following the maturation of certain immune cell types (14, 268). Although several studies have demonstrated the involvement of FFAR2 in inflammation management, these studies showed divergent findings on the potential impact of FFAR2 in inflammatory diseases. Maslowski et al. (203) reported that FFAR2-deficient mice showed deteriorated inflammation in mouse models of arthritis, colitis, and asthma, while germ-free wild-type mice showed correspondingly exacerbated inflammatory conditions. These results clearly show that FFAR2 mediates the beneficial effects of SCFAs, which are present in a high-fiber diet, on the immune system and inflammation. Moreover, using a dextran sulfate-induced colitis mouse model, Masui et al. (204) demonstrated a severe inflammatory condition in FFAR2-deficient mice compared with wild-type mice. Acetate treatment in this model improved inflammatory condition in wild-type mice but not in FFAR2-deficient mice. Moreover, FFAR2 appears to execute a role in inflammatory cell recruitment. Several studies have indicated that FFAR2 is associated with leukocyte migration and cytokine secretion, suggesting that the anti-inflammatory effects of SCFAs are mediated by FFAR2 (14, 57, 295). Indeed, Kamp et al. (142) reported greater intravascular neutrophil rolling and adhesion in FFAR2-deficient mice in response to LPS. Additionally, increased migration of FFAR2-deficient leukocytes into the peritoneal cavity was observed following a challenge with N-formylmethionyl-leucyl-phenylalanine (fMLP). fMLP-induced neutrophil migration after acetate treatment was significantly decreased in wild-type but not in FFAR2-deficient mice, strongly suggesting the involvement of SCFAs in modulating neutrophil migration via FFAR2. In addition, increased migration of neutrophils was observed in wild-type mice fed low fiber compared with wild-type mice fed normal chow (142).

The expression patterns of FFARs on specific tissue-resident leukocytes, such as those in the intestinal lamina propria, remain poorly understood. Nevertheless, FFAR2 expression was found in several leukocytes in the lamina propria of transgenic reporter mice expressing red fluorescent protein (RFP) under the control of the Ffar2 promoter (Ffar2-RFP) (234). A recent study showed that the colonic microbial metabolite butyrate facilitates M2 macrophage polarization in vitro and in vivo. The supernatant from butyrate-treated M2 macrophages increased migration and enhanced wound healing of the mouse epithelial cell lines MLE-12, which were mechanically wounded by scraping a sterile conventional pipette tip across the cell monolayer (137). Additionally, butyrate modulates STAT6 phosphorylation, which is required for M2 macrophage polarization, and histone...
acetylation may play an important role in regulating the level of phosphorylated STAT6 and controlling M2 macrophage polarization (137). Therefore, using FFAR2-deficient mice may enable the dissection of the precise mechanisms underlying the effect of butyrate on histone acetylation/STAT6 signaling in the polarization of M2 macrophages. In addition, Nakajima et al. (222) found that FFAR2 activation by SCFA resulted in induction of the pro-inflammatory cytokine TNF-α in anti-inflammatory M2-type macrophages within the adipose tissue. In contrast, this effect was not observed in inflammatory M1-type macrophages, suggesting a macrophage type-dependent difference in the functions of FFAR2 (222). In addition to the innate immune system, SCFA-FFAR2 signaling was recently shown to be involved in the regulation of adaptive immunity. Wu et al. (362) reported that acetate promoted intestinal IgA response via FFAR2, and that FFAR2-deficient mice had lower levels of intestinal IgA and IgA-positive gut bacteria, compared with those of wild-type mice. Furthermore, acetate-fed wild-type but not FFAR2-deficient mice showed increased intestinal IgA response independent of T cells. Moreover, acetate promoted B cell IgA class switching and IgA production in vitro in the presence of wild-type but not FFAR2-deficient dendritic cells (DCs) (362). In addition, deficiency of FFAR2 resulted in reduced numbers of inducible regulatory T cells (199, 299). Smith et al. (299) revealed that deficiency of FFAR2 resulted in reduced numbers of inducible regulatory T cells in mucosal tissues. SCFA-FFAR2 interactions in gut epithelial cells were also shown to mediate the protective effects of diet and microbiota by modulating the host immune response. Binding of SCFAs to FFAR2 on colonic epithelial cells has been reported to alleviate colonic inflammation by augmenting NACHT, LRR, and PYD domain-containing protein 3 (NLRC3) inflammasome activation (196). Alternatively, SCFAs recognized by mucosal T cells act directly in the nucleus as histone deacetylase (HDAC) inhibitors, thereby promoting differentiation of peripheral regulatory T cells. This effect of butyrate on regulatory T cells can be explained by increased acetylation of histones H3 and H4 at the Foxp3 gene locus (16, 94). Nevertheless, the relationship between dietary metabolites and regulatory T cells is still in its infancy, and further research is warranted to investigate all metabolites and their receptors to uncover their effects on regulatory T cell differentiation or function. A recent study also demonstrated that SCFAs, produced by gut microbiota as fermentation products of dietary fiber, promoted IL-10 production by Th1 cells, thereby inhibiting pathogenic Th1 cell-induced colitis (310). Unlike suppression of T cell differentiation by SCFA stimulation, which is mediated by HDAC inhibition (16, 94, 250), SCFAs promoted IL-10 production by differentiated Th1 cells via FFAR2. Interestingly, butyrate also affects two CD4+ T cell subsets, cytotoxic T lymphocytes (CTLs) and Tc17 cells (193). These findings support the notion that SCFAs not only optimize the function of regulatory T cells and conventional CD4+ T cells but also modulate the expression of effector molecules in CD8+ T lymphocytes in a context-specific manner.

4. Nervous system

The nervous system is involved in metabolic homeostasis via the sensing of peripheral signal molecules derived from food intake. Recent studies suggest that SCFAs and ketone bodies affect brain function via stimulation of the peripheral nervous system or reconstitution of the immune milieu in the brain (354). Propionate enhances norepinephrine release via an intracellular G protein (Gβγ)-PLC-β-MAPK pathway or by voltage-dependent inhibition of N-type Ca2+ channels in norepinephrinergic sympathetic neurons expressing FFAR2 and FFAR3, whereas β-hydroxybutyrate suppresses sympathetic nervous system activity (157, 190, 361). In addition, Lal et al. (168) showed that butyrate can directly stimulate afferent fibers of the vagus nerve. Afferent nerve responses by the intraluminal administration of sodium butyrate are abolished in vagotomized rats, indicating the involvement of vagal afferents in butyrate responsiveness. These findings demonstrate the functional expression of FFAR2 and FFAR3 in the sympathetic nervous system, providing a potential link between nutritional status and autonomic function. Furthermore, there is ample evidence that SCFAs strongly influence the immune condition in the brain. Peripheral immune cells expressing FFAR2 can migrate to the brain via the blood-brain barrier following reception of SCFAs (84, 354), suggesting that SCFA-FFAR2 interactions are needed for proper brain function. Notably, SCFAs directly cross the blood-brain barrier through the host circulating plasma and regulate microglia function or maturation in the brain (273, 354). Meanwhile, Erny et al. (79) confirmed that FFAR2 is not expressed in microglia or other any neuroectodermal CNS cell types, although FFAR2-deficient mice displayed severely malformed microglia with major alterations of dendrite length, number of segments, branching points, terminal points, and increased cell volumes (34, 79). Therefore, SCFAs may control the activity of microglia through other peripheral myeloid cells expressing FFAR2. Thus FFAR2-dependent effects of SCFAs on host physiological functions greatly expand the CNS, and further experiments are warranted to determine the function of FFAR2 in the nervous system.
**C. Pharmacology**

As mentioned above, FFAR2 stimulation by propionate induces the secretion of GLP-1 and PYY (178). Furthermore, treatment of adipocytes with acetate suppresses lipolysis and glycerol release, whereas FFAR2 activation by intraperitoneal injection of sodium acetate leads to a rapid decrease in plasma FA levels in vivo (98). Detailed exploration of the functions of FFAR2 in disease has previously been challenging owing to the low potency of its endogenous ligands (283). However, several studies have identified and characterized FFAR2-selective compounds (121, 122, 126), which enabled the development of FFAR2-selective antagonists or agonists that prevent its pathophysiological functions. FFAR2 antagonists have also been protected by Euroscreen and Galapagos, with both patents including pharmacological and functional assays (Table 4). GLPG0974, a molecule developed by Galapagos (Mechelen, Belgium), is an orally available small-molecule inhibitor of FFAR2 that has reached phase II clinical trials for the treatment of inflammatory bowel disease. Several ligands have been shown to inhibit lipolysis and increase glucose uptake in isolated adipocytes in a dose-dependent manner (26, 134). Some of these FFAR2 agonists and/or antagonists may achieve therapeutic utility for the treatment of T2D in the near future.

Designing selective FFAR2 modulators requires characterization of its orthosteric and allosteric binding pockets, including the identification of requirements for selective activation of FFAR2 against FFAR3. The presence of two arginines and one histidine has been reported as crucial for SCFA recognition by human orthologues of both receptors (306, 314, 338, 344). SCFAs exert different actions through several pathways (27, 290, 331); therefore, development of selective FFAR2 agonists and antagonists is required to identify the beneficial effects that may be dependent on SCFA binding to FFAR2 and to investigate the therapeutic potential of modulating FFAR2 activity. Furthermore, a study based on pharmacological variation between species orthologues suggested that chemically engineered receptors activated solely by synthetic ligands (Rasps) are a useful alternative for probing receptor function. This study suggested that knock-in transgenesis of this RASSL would enable investigation of FFAR2 function in vivo (125). In contrast, feeding of inulin-type fructans as prebiotics was suggested that FFAR2 expressed by intestinal cells are in FFAR2-deficient mice but not in FFAR3-deficient mice, indicating that FFAR2 expressed by intestinal cells are involved in protection against the development of severe food allergy in mice (318). Dietary fiber is fermented by the gut microbiota to produce SCFAs, and butyrate acts predominantly on small intestinal CD103+ DCs to enhance retinoic acid production from vitamin A by promoting retinaldehyde dehydrogenase (RALDH) 2 activity, which in turn increases oral tolerance by inducing regulatory T cell differentiation. Conversely, lack of dietary fiber promotes production of proinflammatory mediators such as thymic stromal lymphopoietin (TSLP) and IL-33, thereby skewing the mucosal DCs to a T helper 2 (Th2) phenotype, which can be remediated by acetate-FFAR2 binding (318). Moreover, a high-fiber diet may increase certain commensal Clostridia species, which produce high levels of SCFAs by fermentation of dietary fiber in the gut. This results in increased IL-22 production by RORγt and CD4+ T cells, increasing barrier function and inhibiting allergen entry, which thereby increases epithelial integrity (303).

**1. Food allergy**

Recently, by using a mouse model of food allergy, the role of dietary fiber and SCFA receptors (FFAR2 and FFAR3) was investigated (318). Dietary fiber modulated food allergy by modulating the responses of mucosal CD103+ DCs, the principal DC subset involved in generating oral tolerance against food antigens by promoting the differentiation of antigen-specific regulatory T cells (114). Interestingly, this protective effect of dietary fiber was eliminated in FFAR2-deficient mice but not in FFAR3-deficient mice, indicating that FFAR2 expressed by intestinal cells are involved in protection against the development of severe food allergy in mice (318). Dietary fiber is fermented by the gut microbiota to produce SCFAs, and butyrate acts predominantly on small intestinal CD103+ DCs to enhance retinoic acid production from vitamin A by promoting retinaldehyde dehydrogenase (RALDH) 2 activity, which in turn increases oral tolerance by inducing regulatory T cell differentiation. Conversely, lack of dietary fiber promotes production of proinflammatory mediators such as thymic stromal lymphopoietin (TSLP) and IL-33, thereby skewing the mucosal DCs to a T helper 2 (Th2) phenotype, which can be remediated by acetate-FFAR2 binding (318). Moreover, a high-fiber diet may increase certain commensal Clostridia species, which produce high levels of SCFAs by fermentation of dietary fiber in the gut. This results in increased IL-22 production by RORγt and CD4+ T cells, increasing barrier function and inhibiting allergen entry, which thereby increases epithelial integrity (303).

**2. Type 1 diabetes**

A recent study has strongly suggested the involvement of gut microbiota and their products in type 1 diabetes (T1D) pathogenesis in nonobese diabetic (NOD) mice and identified the role of FFAR2 in disease progression (199). NOD FFAR2-deficient mice displayed increased islet inflammation and showed fewer islets with no infiltration, indicating that FFAR2 is the molecule responsible for protection from islet β-cell destruction (199). However, unlike normal NOD mice, NOD FFAR2-deficient mice showed only a partial delay in diabetes progression when fed a HAMSA diet containing high acetate content. Moreover, these mice had fewer infiltrated islets compared with that of normal chow-fed NOD FFAR2-deficient mice. Compared with their littermates, NOD FFAR2-deficient mice had significantly reduced numbers of regulatory T cells and higher numbers of IgM+ B220+ B cells in the spleen and pancreatic lymph.
nodes. HAMSA diet led to increased regulatory T cells and decreased autoreactive T cells in NOD. Ffar2^{+/+} mice but not in their NOD Ffar2^{-/-} littermates (199).

3. Cancer

In 2010, Hatanaka et al. (109) reported the transformation activity of Ffar2 in fibroblasts and showed that Ffar2 transcript and protein levels were increased in gastric and colorectal cancers. Conversely, Tang et al. (326) demonstrated that Ffar2 expression was markedly decreased in human colon adenocarcinomas and that Ffar2 mRNA was present in only one of the nine established human colon cancer cell lines. Interestingly, Ffar2 overexpression in the colon cancer cell line HCT18 enhanced the propionate or butyrate-induced antiproliferative effects (326). The gut microbiota has been suggested to partially play a role in the onset and progression of colon cancer (274). In contrast, butyrate and other SCFAs affect the cell cycle by inhibiting proliferation and inducing differentiation and cell death (290, 325, 326). Similarly, another study demonstrated that Ffar2-deficient mice show a severe phenotype in the dextran sulfate sodium model of colon cancer; Ffar2-deficient mice had a considerably high number of tumors, and the individual tumors were much larger in the colons (196). Additionally, the fermentation of inulin-type fructans to propionate counteracted Ba/F3 malignant cell proliferation in the mouse liver; this inhibition of cancer cell growth was Ffar2 dependent (27). Moreover, Ffar2 activation can manage cellular stress through p38 MAPK activation in MCF7 breast cancer cells (368). These findings demonstrate the critical role of Ffar2 in maintaining a healthy composition of gut microbiota leading to suppression of intestinal carcinogenesis, thereby Ffar2 signaling could be utilized as a potential target for therapeutic correction of gut microbiota to suppress intestinal carcinogenesis.

4. Others

Gout is a form of inflammatory arthritis caused by NLRP3 inflammasome activation and IL-1β release triggered by monosodium urate crystals (MSU), which are deposited in joints, tendons, and surrounding tissues (159). Several GPCRs have been reported to regulate MSU-induced NLRP3 inflammasome activation in murine models of gout. It has also been shown that metabolites derived from the murine gut microbiota exacerbate MSU-induced NLRP3 activation and gouty inflammation via Ffar2 (342). Furthermore, a recent study involving a combination of genetic, chemical loss- and gain-of-function approaches revealed that Ffar2 is a critical sensor for butyrate and propionate and suggested a novel role for the SCFA-Ffar2-ERK-NLRP3 axis in mitigating graft versus host disease (92).

The gut microbiota affects microglial maturation and function. Microglia not only respond to local signals within the brain but also receive input from the periphery, including the gastrointestinal tract. Recent preclinical findings also suggest that the gut microbiota plays a pivotal role in regulating microglial maturation and function, and an altered microbial community composition has been reported in neurological disorders with known microglial involvement in humans. Collectively, these findings suggest that bidirectional crosstalk between the gut and the brain may influence disease pathogenesis (1). Compared with conventional gout, germ-free mice show increased numbers of immature microglia across the gray and white matter of the cortex, corpus callosum, hippocampus, olfactory bulb, and cerebellum (79). Consistent with the role of SCFAs in mediating the effects of gut microbes on brain microglia, conventionally colonized Ffar2-deficient mice exhibited microglial abnormalities similar to those observed in microbiota-depleted mice. As microglia do not express Ffar2 directly, whether the effects of the microbiota on microglia are mediated by SCFA signaling remains unclear (34). Notably, germ-free-associated alterations in microglial morphology, abundance, and gene expression patterns are normalized via postnatal supplementation with SCFAs, suggesting that SCFA-producing bacterial species may restore the microglial abnormalities observed in germ-free or antibiotic-treated mice. Further studies are essential to identify the different factors involved in this complex communication network.

VII. GPR41/FFAR3

A. Signaling and Affinity

FFAR3, which has been reported to contribute to the regulation of energy homeostasis (279, 363), was deorphanized in 2003 and identified as a receptor for SCFAs (39, 174). FFAR3 is activated by SCFAs such as propionate (C3), butyrate (C4), and valerate (C5), which are produced by the bacterial fermentation of dietary fiber in the colon (39, 174) (TABLE 3). However, FFAR3 shows a stronger response to the longer SCFAs such as valerate and caproate (C6) than that shown by Ffar2 (39, 174). FFAR3 stimulation with SCFAs inhibits cAMP production and promotes ERK1/2 phosphorylation. Furthermore, these responses are inhibited by pertussis toxin treatment, indicating that FFAR3 is coupled to Gi/o (33, 129) (FIGURE 1). Furthermore, several synthetic compounds have been discovered as FFAR3 agonists or antagonists. Arena Pharmaceuticals have reported a selective agonist (Compound 1) and antagonist (Compound 2) for FFAR3 (180). Schmidt et al. (284) also reported a small carboxylic acid (Compound 3), containing the bulky structure of cyclopropane, that exerts a 100-fold selectivity for FFAR3 relative to Ffar2. In addition, it has been reported that FFAR3 is expressed in various tissues of the body, including adipose tissues, intestines, peripheral nervous system, and immune cells, and that it mediates systemic energy homeostasis via SCFA-induced signaling.
Interestingly, a recent study involving proximity ligation assays, bimolecular fluorescence complementation (BiFC), and fluorescence resonance energy transfer (FRET) revealed that the FFAR2-FFAR3 heteromer displays signaling that is distinct from its parent homomers. The heteromer displays enhanced intracellular Ca\(^{2+}\) signaling (1.5-fold increase against homomeric FFAR2) and β-arrestin-2 recruitment (30-fold increase against homomeric FFAR3) but loses its ability to inhibit cAMP production (15). Although the combination of FFAR2 and/or FFAR3 exerts a different signaling pattern, its precise functions and roles remain to be understood. Therefore, further studies are required to explore the role of these receptors in pathophysiological functions and understand whether receptor heteromerization is necessary for their unique signaling or function.

**B. Physiological Functions**

1. **Adipokine secretion**

FFAR3 activation by propionate (C3) leads to the release of leptin by adipose tissues. In addition, the suppression of FFAR3 mRNA by siRNA decreased leptin secretion by adipose tissues, whereas the overexpression of exogenous FFAR3 increased leptin secretion (8). Another group reported that the propionate-dependent increase in leptin secretion can be inhibited by pretreatment with the G\(_{i/o}\) protein inhibitor pertussis toxin (370). However, other groups were unable to detect FFAR3 expression in mouse adipose tissue by qRT-PCR or in situ hybridization analysis (98, 119, 157). In contrast, other groups found that mouse adipose tissues express FFAR2, but not FFAR3 (98, 119, 327). Zaibi et al. (370) showed that acetate, but not butyrate, stimulates the release of leptin by mesenteric adipocytes in mice. FFAR3 is activated by both acetate and butyrate with equal potency, whereas FFAR2 is activated by acetate, rather than butyrate (370). These findings indicate that SCFA-stimulated leptin secretion is mediated via FFAR2. To robustly clarify these discrepancies in findings, the generation of adipose tissue-specific FFAR2- or FFAR3-deficient mice is necessary. Bellahcene et al. (25) demonstrated the phenotypic differences between male and female FFAR3-deficient mice; compared with female FFAR3-deficient mice, male FFAR3-deficient mice showed higher body fat mass as well as plasma leptin and glucose levels. These differences can be explained by the effect of sex hormones on metabolic regulation in the central or peripheral nervous systems and on adipose tissue distribution.

2. **Gut hormone secretion and immune system**

FFAR3 expression has been confirmed in several types of cells in the gut (133, 279, 328). Using in situ hybridization analysis, Samuel et al. (279) showed that FFAR3 is ex-
pressed by enter endocrine cells. Compared with wild-type mice, FFAR3-deficient mice showed decreased body weight and fat pad weight, but this difference was eliminated in germ-free mice, indicating that the function of FFAR3 depends on the SCFAs produced via dietary fermentation of fiber by the gut microbiota (279). FFAR3 is also expressed by PYY-containing enteroendocrine L cells (264). Samuel et al. (279) showed that GLP-1 and PYY secretion was reduced in FFAR3-deficient mice, although FFAR3 deletion did not affect the amount of chow consumed by the mice. The authors suggested that the decreased PYY levels in FFAR3-deficient mice increase gut motility, which leads to reduced SCFA absorption and consequently a lean phenotype. FFAR3 is necessary for SCFA-mediated secretion of GLP-1 as well as PYY (231, 242), and selective FFAR3 agonists induce GLP-1 secretion from colonic crypt cultures. Additionally, the effects of another synthetic compound AR420626, which has a basic structure similar to that of compound 1, on GLP-1 secretion from colonic crypt cultures was confirmed by stimulation of FFAR2 or FFAR3 using specific synthetic ligands. AR420626 also resulted in IP3 accumulation in COS-7 cells transiently expressing FFAR3 activity (157).

Decreased inflammatory responses were observed in FFAR3-deficient mice compared with wild-type mice. Furthermore, propionate treatment increased energy expenditure and heart rate in adult wild-type mice, which were not observed in FFAR3-deficient mice. Moreover, the effect of propionate on heart rate was suppressed by pretreatment with a β-adrenergic receptor blocker. These observations suggest that propionate activates the sympathetic nervous system (SNS) via FFAR3 (157). Propionate increases the release of noradrenaline from sympathetic neurons, and β-hydroxybutyrate inhibits FFAR3 activity (157). β-Hydroxybutyrate, which is biosynthesized in the liver in response to a low-carbohydrate diet, suppresses propionate-induced sympathetic activation in both primary cultured sympathetic neurons and mice (157). These results indicate that the biological functions of FFAR3, such as SNS activation by propionate and SNS inhibition by ketone bodies, may be involved in the recognition of energy conditions in the body and may thereby contribute to the maintenance of energy homeostasis. FFAR3 also enhances insulin sensitivity through the gut-brain neural circuit, which involves activation of the peripheral nerve FFAR3 by SCFAs produced by gut microbiota from dietary fibers (65). FFAR3 is also expressed in mouse and human pancreatic β-cells as well as in the murine pancreatic β-cell line MIN6 and human pancreatic β-cell line EndoC-βH1, suggesting that it directly regulates insulin secretion (323). Thus SCFAs exhibit beneficial effects on host metabolism via FFAR3 through the peripheral nervous system and insulin secretion in pancreatic β-cells. Recently, a transgenic mouse study using monomeric RFP as a reporter for FFAR3 revealed that FFAR3 is highly expressed in a large population of enteric neurons of both the submucosal and the myenteric nerve plexus, including vasoactive intestinal peptideergic secretomotor neurons (234). Moreover, purified FFAR3-positive cells exhibit higher levels of neuropeptide and neuroendocrine hormone precursors (233), indicating an association between SCFAs and neuropeptide production. Similarly, FFAR3 was also expressed in afferent vagal neurons in the same FFAR3-RFP reporter mouse.
Nøhr et al. (233) demonstrated FFAR3 expression in the nodose ganglion and in sympathetic ganglia; however, a small frequency of FFAR3-RFP-positive neurons in the nodose ganglion was observed, suggesting that nutrient- and gut microbiota-derived metabolites mainly affect afferent vagal nerves indirectly via stimulation of gut hormones (77). To elucidate the precise mechanism of FFAR3 in the nerve, conditional FFAR3-deficient mice or specific ligands to discriminate between FFAR3 expressed in the nerve and other tissues are required.

Recent reports have shown that FFAR3 activation by propionate improved glucose tolerance via the gut-brain neural circuit (65). De Vadder et al. (65) found that FFAR3 is expressed by the nerve fibers of the portal vein and that improved glucose tolerance was observed in SCFA-enriched diet-fed rats; compared with standard diet-fed rats, this effect was eliminated by periportal nervous deafferentation with capsaicin. In addition, treatment with β-hydroxybutyrate, a FFAR3 antagonist, induced a slight decrease in glucose-6-phosphatase activity (65). Moreover, propionate exhibited protective effects on the blood-brain barrier by binding to FFAR3 on the surface of endothelial cells in a recent study (123), indirectly supporting the notion that SCFAs, as gut-derived microbial metabolites, may be crucial mediators in the gut-brain connection.

4. Insulin secretion

As mentioned above, FFAR3 is expressed in mouse and human pancreatic β-cells, and FFAR3 and FFAR2 signaling directly mediates glucose-stimulated insulin secretion via a G_{i/o}-dependent pathway (323). However, little is known regarding the physiological role of FFAR3 in the function of pancreatic β-cells. A recent study reported that FFAR3-overexpressing transgenic and FFAR3-deficient mice showed complementary changes in glucose tolerance, without any significant effects on insulin sensitivity (233). In accordance with previous results (38, 261, 323, 341), in vitro islet incubation assays showed that FFAR3-overexpressing transgenic islets display impaired GSIS, whereas FFAR3-deficient islets show increased insulin secretion. These findings indicate that SCFAs execute FFAR3 signaling in pancreatic β-cells and may play a key role in fine-tuning insulin production to maintain metabolic homeostasis. As GPCR s are important pharmaceutical targets against diabetes (360), further consideration of the action of FFAR3 antagonists as a novel mechanism to enhance glucose-stimulated insulin secretion and as a viable diabetes treatment approach is needed.

5. Immune system

FFAR3 expression is also observed in the spleen and in some immune cells involved in innate and adaptive immunity (174). SCFAs such as propionate can have a profound effect on systemic innate cells and emphasize the strong association between dietary fiber intake and many types of DC- or macrophage-mediated immune responses. FFAR3 signaling by SCFAs has been shown to alter bone marrow hematopoiesis, which subsequently affects Th2 cell development and airway inflammation in models of allergic disease (334). Trompette et al. (334) confirmed the role of propionate and its receptor FFAR3 in the generation of macrophage and DC precursors and in the settling of the lungs by DCs, which have high phagocytic activity but an impaired ability to induce Th2 cell responses. As mentioned above, SCFAs can also modulate cellular function by altering the acetylation pattern of gene promoters, an epigenetic mechanism independent of GPCR activation. SCFAs inhibit HDACs (346), the enzymes that remove acetyl groups from histones, and enhance acetylation of lysine residues in histones, consequently increasing gene transcription and induction of regulatory T cells. In addition, butyrate induces the differentiation of regulatory T cells in vitro and in vivo and ameliorated the development of colitis induced by adoptive transfer of CD4^{+} CD45RB^{hi} T cells in Rag1-deficient mice (94). Although SCFAs are also known to regulate inflammatory responses via FFAR2 (203), this is unlikely to be the case for the effect of butyrate on regulatory T cell differentiation, because acetate, a potent FFAR2 ligand (38), failed to suppress T cell-dependent experimental colitis or induce regulatory T cell differentiation (94). In addition, FFAR2 expression is restricted to myeloid cells among the hematopoietic cell lineages. In contrast, stimulation of naïve T cells by TGF-β1 and butyrate supplementation, an environment favorable for the formation of regulatory T cells, enhanced histone H3 acetylation in the promoter and conserved the noncoding sequence regions of the Foxp3 locus (94). The authors suggested that butyrate can stimulate other FFARs, including FFAR3, on myeloid cells such as DCs to facilitate regulatory T cell differentiation. In addition, Nakajima et al. (221) demonstrated that the maternal microbiome influences regulatory T cell differentiation in the thymus of the offspring via FFAR3-mediated autoimmune regulator (Aire) expression. The offspring of HFD-fed mice exhibited higher expression of Aire, a transcription factor expressed in the thymic microenvironment, suggesting that SCFAs promote regulatory T cell differentiation via increased Aire expression. Notably, FFAR3, the butyrate receptor, is highly expressed in the thymic microenvironment, and Aire expression is not increased after stimulation with butyrate in FFAR3-deficient mice (221). Furthermore, another group reported that the metabolic and functional changes in CD8^{+} T cells are in part mediated by SCFAs via FFAR3 (333). Although the anti-inflammatory properties of SCFAs can prevent protective immunity, fermentable dietary fiber increased the survival of influenza-infected mice. Moreover, addition of butyrate to CD8^{+} T cells isolated from control diet-fed wild-type, FFAR3-deficient, and FFAR2-deficient mice revealed that...
the increase in mitochondrial mass and surface GLUT1 expression was partially dependent on FFAR3, which resulted in a less activated phenotype (333). These findings indicate that SCFAs derived from dietary fiber protect against chronic inflammation and prevent viral infection via FFAR3.

C. Pharmacology

Kimura et al. (157) reported that FFAR3 contributes to energy homeostasis via the modulation of sympathetic activity. Although FFAR2 and FFAR3 are frequently coexpressed by the same tissues, such as enterocytes of the islets, the mechanisms underlying the contribution of these receptors to energy regulation and their interaction with each other remain unclear (133, 144, 233). To robustly determine the potential of FFAR3 as a therapeutic target in T2D, development of FFAR3-selective ligands is necessary. Similar to FFAR2, FFAR3 is also expressed in the intestine and stimulates PYY secretion. In addition, Tolhurst et al. (331) showed that glucose-stimulated GLP-1 secretion in FFAR3-deficient mice was lower than in wild-type mice. This finding was confirmed by Nøhr et al. (233) using an FFAR3-selective agonist, AR420626. Considering these observations, FFAR3 stimulation by selective ligands may be useful for the treatment of metabolic disorders such as T2D.

Recent clinical studies also showed that significantly increased postprandial circulation levels of PYY are likely to be important factors in appetite reduction following Roux-en-Y gastric bypass (RYGB) (254). RYGB is a surgical therapy that has been highly effective for consistent weight loss, normalizing insulin sensitivity, and changing eating habits (197). Moreover, mRNA expression levels of FFAR3, but not FFAR2, increased in the mouse colon after RYGB compared with those in obese mice, suggesting that an altered luminal environment can also drive molecular changes (254). Recent evidence shows that RYGB induces persistent restructuring of the indigenous gut microbiota, which in turn aids the effectiveness of RYGB. Thus gut microbiodeproduced SCFAs may contribute to anorectic hormone secretion via FFAR3. Indeed, the intestinal transit rate and SCFA content in the feces of FFAR3-deficient mice were higher than in the feces of wild-type mice. After colonization of germ-free mice with specific microbes, wild-type mice, but not FFAR3-deficient mice, showed increased PYY levels. These results indicate that SCFA production by bacterial fermentation is essential for the activation of FFAR3 function and that PYY secretion of gut motility is important for SCFA absorption.

FFAR3 may also be a key player in airway inflammation. The gut microbiota composition, fiber content of the diet, and subsequent SCFA production can strongly affect the immune response in lungs and have been suggested to regulate inflammation during induced allergic asthma. Zaiss et al. (371) showed that intestinal helminth infection causes changes in commensal communities, resulting in an increase in SCFAs and reduction of allergic asthma in an FFAR3-dependent manner. In addition, airway DCs, which express FFAR3, showed a less activated phenotype in mice fed a high-fiber diet. Conversely, mice fed a low-fiber diet showed that airway DCs were more activated compared with those from mice fed a control diet. Moreover, consumption of propionate reduced antigen presentation on DCs owing to the FFAR3-dependent modulation of hematopoiesis and attenuated allergic airways disease in a mouse model (334). Additionally, a clinical study also revealed that anaerobes produce high amounts of SCFAs, which were present in the bronchoalveolar lavage of people with cystic fibrosis (CF). SCFAs induced a dose-dependent and pertussis toxin-sensitive IL-8 response in the bronchial epithelium; this response was higher in CF than in normal bronchial epithelial cells. This effect coincided with increased FFAR3 expression in CF than in normal bronchial epithelial cells; this increase in FFAR3 expression was intrinsically driven by lack of cystic fibrosis transmembrane conductance regulator (CFTR) activity and endoplasmic reticulum stress, in addition to the proinflammatory milieu of the CF airways (213). These studies provided a novel insight into the model of diet-microbiome-mediated immune tolerance by circulating metabolites acting on the inflammatory cells and showed that this mechanism influences lung sensitivity to the development of allergic inflammation.

Furthermore, FFAR3 regulates blood pressure via renin release (257), suggesting a broader role for SCFA agonists in homeostasis. Pluznick et al. (257) reported that Olf78 and FFAR3 are expressed in the smooth muscle cells of small resistance vessels. Interestingly, elevation of blood pressure was observed in both Olf78- and FFAR3-deficient mice under antibiotic treatment, which reduced SCFAs derived from gut microbial fermentation. Thus we should consider Olf78 as a novel type of SCFA with therapeutic potential for blood pressure modulation (257). These studies suggest that endothelial FFAR3 lowers baseline blood pressure, likely by decreasing the active vascular tone without altering passive characteristics of the blood vessels. They also suggest that FFAR3-deficient mice exhibit hypertension of a vascular origin.

Recently, Thirunavukkarasan et al. (329) showed that FFAR2 stimulation by SCFAs can activate LATS1 (resessor of YAP1), which results in an increase in E-cadherin levels. In contrast, FFAR3 activity inhibits the MAP/ERK pathway. Cumulatively, these results demonstrate that SCFA-FFAR2/FFAR3 signaling can reduce the invasive potential of breast cancer cells via multiple signaling mechanisms, including the regulation of proliferative pathways, cytoskeletal organization, and expression of adhesion proteins (329).
VIII. OTHER GPCRs FOR FFAs

FFAs derived from diet and metabolites also act as signaling molecules through other GPCRs. These are discussed below.

A. GPR109A

GPR109A/HCA2 was initially identified as a niacin receptor that is also activated by ketone body β-hydroxybutyric acid and the SCFA butyric acid, but not by acetate and propionate (4). The EC50 value for GPR109A by butyrate is ~0.7 mM (4). GPR109A is a coupled Gs protein and is expressed in colonic epithelial cells; however, the expression is reduced in germ-free mice in the absence of gut microbiota (60). Butyrate-mediated GPR109A activation suppresses colonic inflammation and carcinogenesis by promoting anti-inflammatory properties via macrophages and DCs and induces the differentiation of IL-10-producing and regulatory T cells in the colon (296). Additionally, GPR109A is also expressed in adipose tissues and regulates lipid metabolism by regulating adipose macrophages (4, 80, 191, 297).

B. GPR170 and GPR31

GPR170, also known as oxoeicosanoid receptor 1 (OXER1), and GPR31 are most closely related in amino acid sequence to GPR170 (103, 120, 139). GPR170 is coupled with the Gs protein and is the receptor of a family of arachidonic acid metabolites such as 5-hydroxyeicosatetraenoic acid (5-HETE) and 5-oxoicosanoic acid (5-oxo-ETE). However, GPR31 is the receptor for a different arachidonic acid metabolite, 12-hydroxyeicosatetraenoic acid (12-HETE), and is coupled with the Gi protein. GPR170 is highly expressed by human white blood cells, particularly eosinophils, and to a lesser extent neutrophils, basophils, and monocytes. GPR31 is highly expressed in the PC-3 prostate cancer cell line. GPR170 and GPR31 activate the MEK-ERK1/2 signaling pathway, but unlike GPR170, GPR31 activates NF-κB and does not trigger an increase in [Ca2+]i (103). As these receptors are activated by arachidonic acid metabolites, they may be involved in inflammatory and allergic responses. GPR31 also functions to induce dendrite protrusion in intestinal CX3CR1+ cell as a receptor for gut microbial pyruvic and lactic acids (216). However, the precise functions of GPR170 remain unclear.

C. GPR132

GPR132/G2A is classified as a member of the proton sensing GPCR subfamily. GPR132 is a receptor for several LA metabolites, such as 9-hydroxyoctadecanoidic acid (9-HODE) and 13-HODE (235, 272), and the arachidonic acid metabolites, including 5-HETE, 12-HETE, and 15-HODE (235). GPR132 mediates the increase in [Ca2+]i by coupling with both Gi and pertussis-toxin-sensitive Gq protein. GPR132 activation by 9-HODE blocks cell proliferation by promoting the secretion of IL-6, IL-8, and granulocyte-macrophage colony stimulating factor in keratinocytes (235). Therefore, it is suggested that 9-HODE acts in the human skin to block the proliferation of damaged cells by triggering the secretion of these cytokines (235). Moreover, GPR132-deficient mice develop a slowly progressive wasting syndrome characterized by lymphocytic infiltration into various tissues (173).

D. GPR84

GPR84 is an MCFA receptor for undecanoic acid (C11) and lauric acid (C12) (350). GPR84 is coupled with the pertussis toxin-sensitive Gi/o protein and is predominantly expressed in the bone marrow as well as the lungs and peripheral leukocytes (172, 350). In particular, GPR84 expression is increased in LPS-induced activated macrophages and neutrophils. In addition, MCFA stimulation led to the production of the IL-12 p40 subunit in RAW264.7 cells, the mouse monocyte/macrophage cell line (350). T cell stimulation by anti-CD3 increased IL-4 but not IL-2 or IFN-γ production in GPR84-deficient mice (339). GPR84 was up-regulated by adipogenesis induction in 3T3-L1 preadipocytes and in the adipose tissue of obese patients and HFD-induced obese mice (217). TNF-α released by GPR84 mediated-infiltrating macrophages in adipose tissue aggravates the pathogenesis of obesity and T2D. Additionally, although GPR84 deletion had no effect on body weight and glucose tolerance in MCFA diet (coconut oil-rich)-fed mice, it resulted in increased hepatic triglyceride production (74). Thus GPR84 plays an important role in immune and metabolic responses and may mediate the crosstalk between immune cells and adipocytes.

E. GPR119

GPR119 is mainly expressed in enteroendocrine cells and pancreatic β-cells. Oleoylethanolamide, a monounsaturated analog of the endocannabinoid produced in the small intestine, is an endogenous ligand for GPR119 and reduces food intake and body weight gain via GPR119 in rats (245). In addition, GPR119 is activated by 2-monocacylglycerols such as 2-palmitoylglycerol, 2-linoleoylglycerol, and 2-oleoylglycerol (105, 236, 300). GPR119 can promote insulin secretion by Gsα activation via GSIS induction in pancreatic β-cells directly and the secretion of incretins such as GLP-1 and GIP in enteroendocrine cells indirectly (53, 54, 170).

F. Olfr78

Olfr78 is an olfactory receptor that was identified as an SCFA receptor for acetate and propionate but not for bu-
tyrate (257). The EC_{50} values for acetate and propionate are 2.35 mM and 920 μM, respectively. Olfr78 is coupled with G_{q} protein and is expressed in the renal vessel involved in renin secretion in the blood vessels. Thus gut microbial SCFAs regulate blood pressure by modulation of renin secretion via Olfr78 (227, 257, 258). Olfr78 is also expressed in enteroendocrine cells of the colon (85). Therefore, although the metabolic effects of this SCFA receptor are unclear, it is speculated to be involved in energy metabolism via the endocrine system.

IX. CONCLUSIONS

Recent evidence shows that various metabolic and immunological disorders have been linked to diet and gut microbiota composition. Several studies have proven that FFARs are nutrient sensors expressed in various tissues and cells and that they regulate both energy metabolism and immune response (TABLE 1).

FFARs were originally identified as receptors for FFAs, and thus significant quantitative evidence is necessary to understand the various physiological functions of FFARs. The ligands show wide-ranging metabolites because FFARs are activated primarily by FFAs, which are derived from food and the corresponding digestion products or products of microbial fermentation in the gastrointestinal tract. Moreover, although the affinities are different, several FFAs can activate the same FFAR, whereas one FFA can activate several FFARs. In vitro and in vivo studies have shown that the physiological functions of FFARs ultimately contribute to the regulation of metabolic energy and the immune system (FIGURES 5–8). However, these FFARs also exhibit overlapping functions via similar signaling pathways involving the activation of [Ca^{2+}], cAMP, or ERK1/2 responses via G protein (G_{q} or G_{i/o})-dependent or G protein-independent pathways per the reported physiological function of FFARs. Moreover, the expression patterns and functions of each FFAR have been reported in the same several tissues. These lines of evidence demonstrate a striking feature of FFAs and FFARs, which is their redundancy; that is, there are several FFAs and FFARs in an organism that keep receptor activation maintained at a certain level. Changes in nutritional states, such as diets, fasting, and obesity, can alter the ratio and amounts of FFAs; thus maintaining the activation levels of each FFAR via redundancy may represent optimal fine-tuning for maintaining homeostasis. Therefore, FFARs maintain body homeostasis by regulating biological processes and sensing nutritional states; FFARs are also important for acting as modulators rather than initiators of biological processes. This feature of FFARs can thus explain several inconsistent reports regarding FFAR physiological function as described in this review.

Some aspects regarding the physiological functions of FFARs remain unclear. Understanding the relative contribution of each FFAR to the regulation of metabolic energy and immune response within the whole body may provide insights into the mechanisms underlying the maintenance of body homeostasis through diet. Moreover, beneficial results were obtained for synthetic compounds of FFARs during early clinical trials, suggesting that future research will increase the therapeutic potential of FFARs. It has also been well established that the gut microbiota deeply impacts host energy metabolism and inflammatory response. The discovery of FA receptors as receptors for gut microbial metabolites may provide molecular links to further explain these interactions.

In conclusion, further analysis of FFARs may be important to better understand nutritional sensing processes and develop therapeutic compounds for treating immune diseases and metabolic energy disorders such as obesity and T2D. Although the involvement of FFARs in the beneficial effects of specific diets remains unclear, unraveling the mechanisms underlying the relationship between diet and health may lead to novel therapeutic strategies.

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