G-PROTEIN COUPLED RECEPTORS FOR FREE FATTY ACIDS AS NOVEL TARGETS FOR TYPE 2 DIABETES

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Summary

Diabetes mellitus is a chronic illness which has been one of the major world health problems. As prevalence of diabetes is increasing, there is an imperative need to develop novel therapies, as there is no drug till date which can target diabetes as well as its associated complications. One of the novel strategies to treat diabetes mellitus is to target G-protein coupled receptors for free fatty acids. GPR 40-43 is a G protein coupled receptor family, activated by free fatty acids and plays an important role in insulin secretion and insulin resistance, especially GPR40. This review focuses on recent developments in this area.

Key Words: Free fatty acids, GPCR, glucose stimulated insulin secretion, insulin resistance, GPR, Type 2 diabetes

Introduction

Type 2 Diabetes (T2D) belongs to a group of disorders characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins; and an increased risk of complications from vascular disease [1]. People suffering from T2D constitute 71% of total diabetic population. Its prevalence in developing countries is projected to double by 2030. The high prevalence of diabetes is combined with the associated increased mortality and morbidity, primarily as a result of macrovascular and microvascular long-term complications [2, 3]. T2D results from both peripheral insulin resistance and impaired insulin secretion. Insulin resistance arises as a consequence of obesity, a sedentary lifestyle and aging, with resulting hyperglycemia and diabetes, blood pressure elevation and dyslipidemia collectively called 'metabolic syndrome X'.

As mentioned above main feature of T2D is insulin resistance. Insulin is produced by β- cells of islets of pancreas and plays an important role in maintenance of glucose homeostasis of the body. Insulin decreases the blood glucose levels by decreasing its release from and increasing its uptake into various organs, mainly liver, muscle and adipose tissue. Insulin exerts its action by acting on insulin receptors located on the target tissues [1].

In case of T2D the sensitivity of these organs to insulin decreases, exact causes of which are yet to be elucidated. But, two well accepted mechanisms are insulin receptor down regulation and abnormalities of the signaling pathways that link receptor activation with corresponding cellular effects. But, recent studies indicate that G-protein coupled receptors and adipose tissue also play an important role in development of insulin resistance through free fatty acids.

Currently available treatments for T2D include sulfonyl ureas, biguanides and thiazolidinediones. Nevertheless, none of these treatments is completely effective against T2D and its associated complications. Furthermore, these agents have their own side effects like hypoglycemia with sulfonyl ureas and hepatotoxicity with biguanides and thiazolidinediones. In many cases monotherapy gradually fails to improve blood glucose control and hence combination therapy is employed. The long term success of these treatments varies substantially. Thus, there is an imperative need for novel therapeutic approaches for glycemic control that can complement existing therapies and possibly attempt to preserve normal physiological response to meal intake. Many novel targets are proposed but not yet introduced for treatment, like protein tyrosine phosphatase1B and glycogen synthase kinase-3 inhibitors [4]. Some PPAR (Peroxisome Proliferator Activated Receptor) dual agonists (Aleglitazar, Tesaglitazar) are under clinical development.

One of the novel strategies proposed for the treatment of T2D is to target G-protein coupled receptors (GPCRs), which probably represent the largest of all the gene families and on which >30% of the existing prescription drugs act [5]. Human genome codes for 865 GPCRs of which more than 100 are orphan receptors, whose ligands and functions are yet to be discovered [6, 7]. Large scale screening with over 1500 ligands using an intracellular Ca^{2+} sensing assay has led to deorphanization of a GPR 40-43 family [8], for which saturated and unsaturated fatty acids are found to act as ligands. The human genes encoding this family are localized in a cluster on chromosome 19q 13.1. The other deorphanized GPCRs which play a role in glucose homeostasis, regulation of body function and immune function and could be targeted to treat diabetes include GPR120, GPR119 and GPR84.

G protein coupled receptors

GPCRs contain seven transmembrane units composed of single polypeptide chain and are activated by a wide variety of ligand types, including light, amino acids, lipids, peptides and proteins [9]. GPCRs have diverse roles including maintenance of overall homeostasis of the organism, embryo development, learning, memory, vision, smell and taste, energy homeostasis and islet function [10]. Phylogenetic analysis has shown that GPCRs can be clustered into 5 subfamilies as glutamate, rhodopsin, adhesin, Frizzled/Taste2 and secretin families [11]. Human fatty acid GPCRs and the single receptor for fatty acid amides belong to the rhodopsin family.

One such well defined receptor cluster is formed by GPR40-43 which belongs to the subfamily of nucleotide and lipid receptors [13]. The sequence of these receptors is 39% identical. Other recently discovered target receptors for fatty acids and fatty acid amides are GPR 119 [12] and GPR 120 [13] respectively. Receptors of the nucleotide and lipid subfamily are typically activated by negatively charged ligands and are characterized by the presence of basic residues at specific positions within their transmembrane units. GPR40 and GPR120 are activated by medium and long chain fatty acids, whereas GPR43 and GPR41 are activated by short-chain fatty acids. GPR 119 is activated by long chain fatty acid amides such as oleyoyl ethanolamide and lysophosphatidylcholine [10].

GPR40 receptor is coupled to G_q, GPR41 selectively activates G_i whereas GPR43 can activate both G_i and G_q. GPR 119 is coupled to G_s whereas GPR 120 is coupled to G_q. Recent studies indicate that two arginine residues at 5.39 and 7.35 are the interaction sites for negatively charged fatty acid molecules. It was also proposed that the difference in the bulkiness of the residue at 6.38 is responsible for the selectivity of the receptor towards long or short chain fatty acids. GPR 119 and GPR 120 do not show significant similarity to the GPR40 cluster in sequence. GPR 119 belongs to the subfamily of the biogenic amine and MECA (Melanocortin, Endothelin, Cannabinoid and Adenosine) receptors. GPR 120 has no close relatives and belongs to a subfamily, which contains several orphan receptors and a cluster of melatonin receptors.

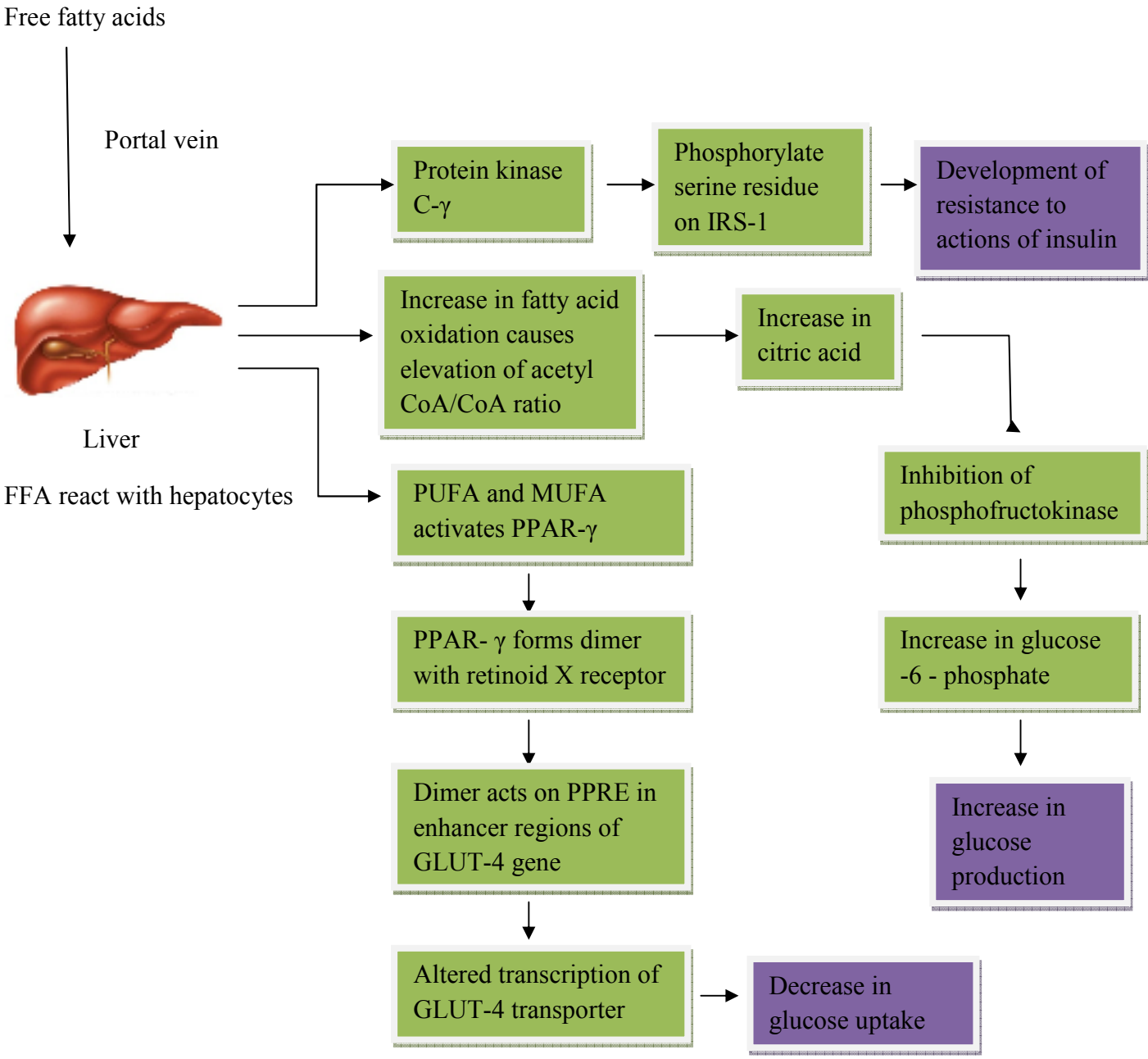
Free fatty acids

Fatty acids are synthesized by the extra mitochondrial system which is responsible for the complete synthesis of palmitate from acetyl-CoA in the cytosol [14]. This system is present in many tissues, including liver, kidney, brain, lung, mammary gland, and adipose tissue. Although the main role of fatty acids is to reserve energy, they play a significant role in insulin utilization by liver and muscle and glucose stimulated insulin secretion (GSIS) from pancreas through GPR40 [14]. Aberration in the process of fatty acid oxidation leads to diseases associated with hypoglycemia. Elevated levels of free fatty acids which are not bound to plasma albumin play an important role in development of insulin resistance and impairment of β cell function, which are the main causes of hyperglycemia.

In order to discuss the role of free fatty acids in insulin resistance, it is important to distinguish between insulin resistance in adipose tissue and subsequent elevation of plasma fatty acids, and mechanisms of free fatty acid induced insulin resistance [15]. In T2D patients the EC_{50} of the insulin (i.e. the insulin concentration that exerts 50% of the maximum effect) increases two to three fold and the target tissues develop resistance to the actions of insulin such as, glucose uptake in muscle, liver, to minor extent in adipose tissue and inhibition of lipolysis in adipose tissue. In adipose tissue it is not the decrease in glucose uptake, but the increase in lipolysis which exerts significant effect on glucose homeostasis. Increase in lipolysis, especially in visceral adipose tissue further releases fatty acids and glycerol, finally leading to increased synthesis of glucose (Fig. 1).

In case of obesity, the fat accumulation in the adipose tissue increases. Excess abdominal adipose tissue has been shown to release increased amount of free fatty acids which directly affect insulin signaling, diminish glucose uptake in muscle, drive exaggerated glucose synthesis and induce gluconeogenesis in liver. A number of mechanisms were proposed to explain the development of insulin resistance caused by elevated free fatty acids. Free fatty acids and hormones released by the visceral adipose tissue enter the portal vein, by which they reach the liver. In the liver they interact with the hepatocytes and immune cells. There fatty acids cause activation of protein kinase C-γ, which phosphorylate serine residue on insulin responsive substrate (IRS-1). This prevents the phosphorylation of IRS-1 on tyrosine residue which is required for activation. This finally leads to insulin resistance and it further leads to decrease in glucose uptake by hepatocytes as well as increase in the production of glucose [16]. Impairment of the glucose uptake by liver has significant effect on blood glucose levels since 40% of the ingested glucose is up taken by liver. Another mechanism which explains decrease in glucose uptake by fatty acids is increased fatty acid oxidation which causes elevation of mitochondrial acetyl-coA/coA and NADH+/NAD+

Figure 1: Role of free fatty acids in development of insulin resistance in liver



FFA: free fatty acids, IRS: insulin responsive substrate, PUFA: poly unsaturated fatty acids, MUFA: mono unsaturated fatty acids, PPAR: peroxisome proliferator activated receptors, GLUT: glucose transporter.

ratios leading to inactivation of pyruvate dehydrogenase. Increased acetyl-coA also causes an elevation of citrate levels (Krebs cycle), leading to inhibition of phospho fructo kinase and accumulation of glucose-6-phosphate, which further proceeds to increased production of glucose. Fatty acid induced elevation in the glucose production can be ascribed to over expression of Glucose-6-phosphatase [17].

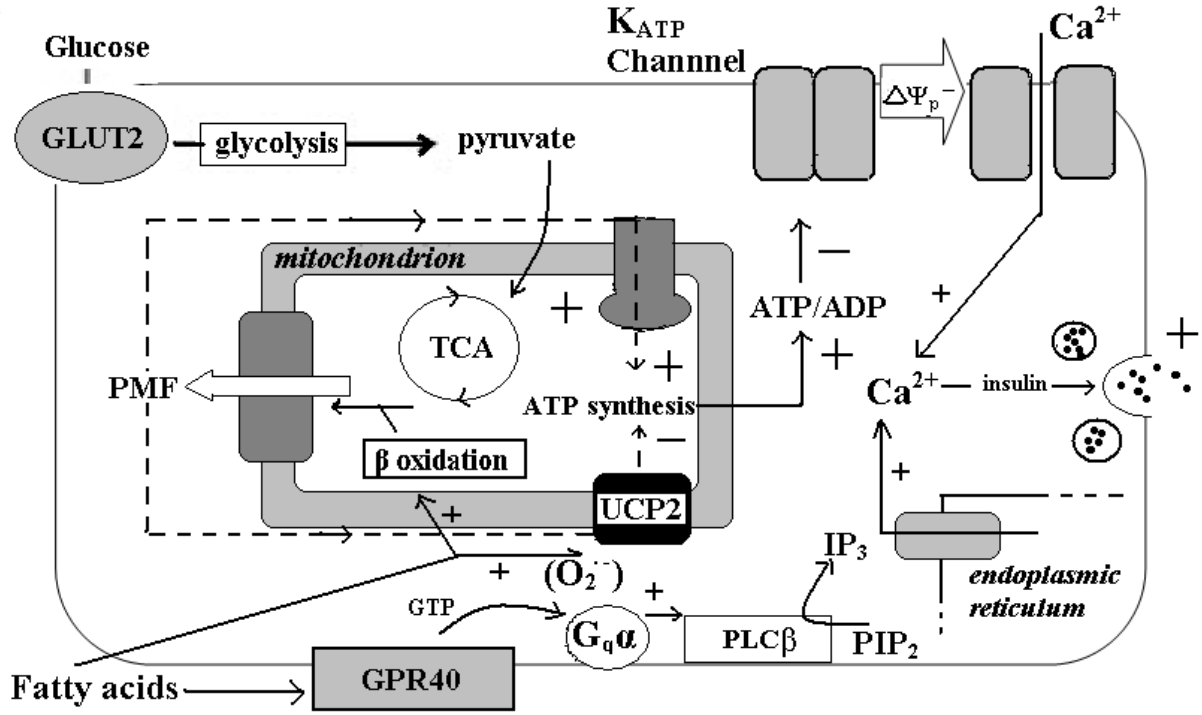
Another mechanism proposed for free fatty acid induced insulin resistance involves the activation of PPARγ [18]. According to this, polyunsaturated fatty acids as well as monounsaturated fatty acids act as ligands for PPARγ receptors. When, free fatty acid molecule binds to the PPARγ receptor, it forms a receptor heterodimer with Retinoid X receptor [19]. This dimer binds to PPAR responsive element (PPRE) in enhancer regions of various genes and alters the regulation of transcription of certain proteins like GLUT-4 which are the main regulators of the glucose uptake. This finally leads to decrease in glucose uptake by liver. Long chain poly unsaturated fatty acids cause less activation, whereas fatty acids with 18-20 carbons cause greater activation of PPARγ [18]. Thiazolidonediones (pioglitazone and rosiglitazone), a class of oral hypoglycemics exert their anti diabetic effect by activating PPARγ receptors.

GPR40: As mentioned earlier free fatty acids play an important role in the control of β cell functions. It is evident from recent experiments that cytosolic free fatty acids play an important role in integration of nutrient secretagogue signals and insulin release [20, 21]. GPR40 is a membrane-bound G-protein-coupled receptor. It is preferentially expressed in pancreatic β cells in rodents and has been shown to be involved in the regulation of GSIS after acute exposure to mid- or long-chain fatty acids in *in-vitro* experiments [22]. In humans also, GPR40 mRNA is expressed in pancreatic β cells [23, 24]. Its expression is 20 fold more in pancreatic islets than pancreas and the level is comparable to that of sulfonyl urea receptor gene [25]. Earlier it was proposed that free fatty acids must be transported across the membrane, where they undergo metabolism and exert their effects [26, 27]. But, deorphanization of GPR40 has proved this assumption wrong [28]. A number of experiments indicate that GPR40 mediates the majority of effects of free fatty acids on insulin secretion (Fig. 2) [29, 30].

Activation of GPR40 receptor by medium or long chain fatty acids results in activation of phospholipase C (PLC). PLC cleaves membrane bound phospholipid, phosphotidyl ionositol biphosphate (PIP2) into IP3 and DAG. IP3 stimulates ryanodine receptors (RYR) on sarcoplasmic reticulum and stimulates the release of calcium into the cytosol and hence increases intracellular [Ca^{2+}]. This increase in intracellular [Ca^{2+}] is responsible for the exocytosis in pancreatic β cells and release of insulin. *Feng et al* have shown that, in pancreatic β cells, linoleic acid decreases the voltage gated current by activating cAMP and protein kinase A through GRP 40 and causes excitation [31]. But, the precise mechanism of activation of cAMP by linoleic acid is not known till now. So, GPR40 acts as a signaling mechanism through which fatty acids regulate insulin secretion [32]. *Chi shing sum et al* reported using site directed mutagenesis that twelve residues within the putative GPR40 receptor binding pocket are responsible for the binding and interaction between the free fatty acid ligands and the receptor. They suggested that free fatty acids are anchored on their carboxylate groups by arginine residues and histidine and tyrosine residues are involved in the hydrophobic and/or aromatic interactions [23].

Recent studies have proved that long chain fatty acids, palmitic acid and linoleic acids increase GSIS in insulinoma (MIN-6 and INS-1E) cell lines. But, the effects are greatly diminished, when the expression of GPR40 is decreased [21].

Figure 2: Insulin secretory pathway of free fatty acid receptors



GLUT: glucose transporter, KATP: ATP dependent potassium channel, PMF: protein motive force, TCA: tricarboxylic acid cycle, UCP: uncoupling protein, PLCβ: Phospholipase C β, PIP_2: phosphatidylinositol biphosphate, IP_3: inositol triphosphate, $\Delta\Psi_p$: plasma membrane potential.

Some recent studies [29, 33, 34] have shown that acute and chronic effects of fatty acids are different and GPR40 mediates both the effects. They have shown that acute elevation of fatty acid levels causes increase in release of insulin from β cells, which lead to hyperinsulinemia, hepatic steatosis, glucose intolerance and increased release of glucose from liver, Whereas, chronic elevation causes deterioration of β cells which is referred to as lipotoxicity and leads to hypoinsulinemia [35]. The molecular mechanism for the hyperinsulinemia on acute exposure is not clear. They have also shown that mice lacking GPR40 gene are protected against hyperinsulinemia, hepatic steatosis, hypertriglyceridemia and glucose intolerance. It was also proved that transgenic mice over expressing GPR40 in β cells develop T2D [35]. So, GPR40 plays an important role in mediating effects of free fatty acids on insulin homeostasis and alteration of its expression has either protecting (decreased expression) or sensitizing (over expression) effects against T2D.

Flodgren et al have described the effects of GPR40 on glucagon secretion. Using double stain techniques they have reported that α-cells (glucagon producing cells) are found mainly in the periphery of the islets and also that GPR40 expression collocates with that of α-cells [36]. They have also shown that GPR40 exerts stimulatory effect on glucagon secretion. *In vitro* studies utilizing hamster glucaganoma cell (In-R1-G9) demonstrated that glucagon secretion is increased in dose dependent manner, when exposed to long chain fatty acids [36]. This effect was paralleled by increase in PIP2 hydrolysis which is the molecular mechanism of GPR40. Also, gain in function mutation of GPR40 has increased the glucagon secretion.

So, free fatty acids have a stimulatory effect on glucagon secretion and this is mediated by GPR40.

Apart from glucose homeostasis, GPR40 receptor plays a significant role in some other physiological processes also. *Sara et al* reported the expression of GPR40 in gastro endocrinal cells and they have shown that GPR40 mediates the free fatty acid induced incretin secretion [37]. GPR40 and related receptors are also involved in the control of cell growth [38] and survival via activation of the ERK and phosphatidylinositol 3-kinaseprotein kinase B (Akt) signaling pathways [39].

GPR 41 and GPR 43: Short chain fatty acids are produced by microbial flora in small intestine [40, 41]. As mentioned earlier, short chain fatty acids act as ligands for GPR41 and GPR43. But, the optimum length of carbon chain to activate GPR43 is one to three, whereas for GPR41, it is three to five. GPR41 is coupled to G_i and GPR43 is coupled to G_i and G_q. GPR41 is 38% identical to GPR43 in amino acid sequence [42]. GPR 42 sequence is 98% identical to that of GPR41 and differs at only six amino acid positions [43]. But, it is not activated by short chain fatty acids. This is due to presence of tryptophan residue instead of arginine in GPR41 at position 174 [7].

GPR43 expression is high in adipose tissue and is increased on feeding with high fat diet and on treatment with troglitazone, whereas GPR41 mRNA is not present in adipose tissue [44]. So, GPR43 may play a role in adipogenesis and adipocyte differentiation and development. In turn GPR41 is highly expressed in brain, lung [45] and mainly blood vessel endothelial cells [46]. GPR43 is also expressed in peripheral blood leukocytes, especially in monocytes and neutrophils and it may play an important role in short chain fatty acid induced chemotaxis of monocytes [47].

When propionic acid is accumulated in the blood, it causes propionic edema (neonatal and infantile ketoacidosis), impairs immune function and makes the person susceptible to opportunistic infections [48]. Also, there is an overlap between distribution of GPR43 and cell types activated by propionic acid. Concentration of propionate which causes immunosupression and propionic edema is high enough to activate GPR43 receptor. So, it is evident that GPR43 may be responsible for immunosupression and propionic edema caused by propionic acid.

Tomo Y et al have shown that mRNAs of GPR41 and GPR43 are expressed in the MCF-7 human breast cancer cell line, with GPR43 expression being notably higher than that of GPR41 [49]. They have shown that acetate, propionate and butyrate have induced an acute increase in $[Ca^{2+}]_i$ in MCF-7 cells in a concentration dependent way and the this increase can be inhibited by silencing GPR43 using corresponding siRNA. They have also shown that this process occurs through activation of p38 mitogen associated protein kinase. *Kimura et al* have reported that GPR41 plays an important role in activation of p53 during apoptosis in ischemic hypoxia and reoxygenation [50].

Table 1: G-protein receptors for free fatty acids- endogenous agonists, G-proteins involved and physiological roles

S.No.	Receptor	G-protein involved	Endogenous agonists	Physiological roles
1	GPR 40	G_q	Medium and long chain fatty acids	Stimulates glucose stimulated insulin secretion Increases glucagon secretion Stimulates incretin secretion Controls cell growth
2	GPR 41	G_i	Short chain fatty acids	Adipogenesis, adipocyte differentiation and development
3	GPR 43	G_i	Short chain fatty acids	Chemotaxis of monocytes Immunosupression.
4	GPR 120	G_q	Medium and long chain fatty acids	Activates GLP-1 Stimulates glucose stimulated insulin secretion Inhibition of apoptosis Adipocyte differentiation and development
5	GPR 119	G_s	Phospholipids and fatty acid amides	Activates GLP-1 Glucose stimulated insulin secretion Decreases food intake

GPR 120 and GPR 119: GPR 120 is an orphan G protein coupled receptor which is abundantly expressed in small intestine and is activated by long chain free fatty acids [13]. GPR 120 is coupled to G_q and activates phospholipase C, when stimulated and causes breakdown of PIP2 into IP3 and DAG. This further leads to increase in intracellular calcium which is responsible for its physiological actions. It activates Glucagon like peptide-1 (GLP-1) and extracellular signal regulated cascade. It shares similar ligand characteristics with that of GPR 40 and indirectly promotes glucose regulated insulin secretion [13, 21]. *Susumu et al* have shown that saturated free fatty acids with a chain length of C14 to C18 and unsaturated free fatty acids with a chain length of C16 to C22 enhance cell survival of serum starved murine enteroendocrine STC-1 cell lines [51]. Free fatty acids which have high potency in this respect include linolenic acid, palmitoleic acid and docosahexaenoic acid. They have shown by RNA interference experiments that GPR 120 is mainly involved in the inhibition of apoptosis by making use of phospho lipase C–ERK and phosphotidyl ionositol-3 kinase pathways.

Chizu et al have reported that GPR 120 is highly expressed in adipose tissue whereas GPR 40 is not present. They have demonstrated that when GPR 120 expression is decreased using corresponding siRNA, the number of lipid droplets and PPARγ2 expression were decreased on high fat diet treatment. So, GPR 120 may be involved in adipocyte development and differentiation [52]. Along with adipocyte differentiation, it is also involved in the expression of adipocyte specific genes such as aP2 and leptin [53]. However precise molecular function of GPR 120 in adipocytes is not clear and hence requires further studies.

GPR 119 is expressed mainly in pancreas and fetal liver, but its distribution in gastrointestinal tract is also reported in some studies [45, 54]. It is coupled to G_s and hence acts by increasing cAMP when stimulated. It increases GSIS when activated by a mechanism similar to that of GPR 40. It also increases GLP-1 peptide secretion in the gut [55]. The endogenous agonists reported for GPR 119 are phospholipids and fatty acid amides. Some studies in mice using a GPR 119 agonist oleoylethanolamide have shown to decrease food intake, as well as to increase GLP-1 secretion [55]. But, synthetic agonists for the treatment of T2D in clinical use are yet to be synthesized.

Conclusion

Initially free fatty acids were thought to be only essential nutritional components, but now it is proved that they can also function as signaling molecules, especially in altering glucose and insulin homeostasis. One of the important mechanisms by which they affect glucose homeostasis is by acting directly on G-protein coupled receptors, GPR40-43 and 120. Especially GPR40, which is abundantly expressed in pancreatic β islets, plays an important role by acting as a modulator of GSIS. GPR40 agonists can be used to treat diabetes, which act by potentiating GSIS. Already research has been done in this area and some GPR40 agonists based on 3-(4-{[N-alkyl]amino}phenyl) propanoic acid were synthesized and proved for affinity towards GPR40 receptor [32]. But, the effectiveness of these compounds in diabetes is yet to be proved and hence require further investigation.

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