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**Diabetes-Induced Expression and Regulation of GLP-1 levels by Bile Acid
Receptors (TGR5 & FXR)**

Masters of Science Thesis submitted by

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ABSTRACT

DIABETES-INDUCED EXPRESSION AND REGULATION OF GLP-1 LEVELS BY BILE ACID RECEPTORS (TGR5 & FXR)

By Joseph Raymond Spengler, M.S.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

Virginia Commonwealth University, 2017.

Major Director: John R Grider, PhD Professor, Department of Physiology

Diabetes Mellitus has continued to drastically affect the health of the world and many complications can prove fatal. As long as this metabolic disease persists, research discoveries will need to continue to be made so that patient outcomes and healthcare are dramatically enhanced. In recent years, GLP-1 has been the topic of conversation for diabetes research, due to its promising effects in promoting insulin sensitivity. Furthermore, bile acids and their receptors (TGR5 & FXR) have shown promise in their actions in the regulation of GLP-1, and thus glucose homeostasis. Here we have shown the detection and increased expression of TGR5 and GLP-1, and decreased expression of FXR in diabetic mouse intestinal mucosa tissues. We have also shown the detection and increased expression of these receptors in STC-1 cells. More importantly we have linked the connection of increased glucose concentration (hyperglycemia) to increased TGR5 activation to increased GLP-1 release, thus leading to increased insulin sensitivity and altered diabetic outcomes.

1. INTRODUCTION

1.1 Diabetes Mellitus

1.1.1 Diabetes General Information

Diabetes is a term most notably used to describe a group of high glucose related metabolic diseases, better known as diabetes mellitus (DM). Among this group of metabolic diseases related to prolonged hyperglycemia are type 1 DM, type 2 DM, gestational diabetes, as well as other diseases and syndromes.¹ Patients with diabetes mellitus can have symptoms including polydipsia, polyphagia, and polyuria.¹⁰ Patients with diabetes can also experience complications including diabetic ketoacidosis, strokes, comas, pedal ulcers, heart disease, and kidney failure.⁶ In many cases, complications from diabetes mellitus can be fatal.¹

Diabetes mellitus affects millions of people in the United States and worldwide.⁶ In addition to genetics and environment, there are many risk factors and predispositions related to diabetes, including race, ethnicity, age, exercise, diet, and levels of hemoglobin glycation\A1C levels.¹

1.1.2 Type 1 Diabetes Mellitus

Insulin-dependent diabetes mellitus (IDDM), also known as type 1 diabetes, is an autoimmune disease in which the pancreas fails to produce enough insulin.^{1,10} The pancreas has both endocrine and exocrine functions. Its exocrine function consists of the production and release of digestive enzymes that aid in the absorption of nutrients in the small intestine. The

endocrine function of the pancreas consists of the production and release of hormones from the specialized group of cells, known as the Islets of Langerhans. The main cell types in these pancreatic islets are the alpha and beta cells. Alpha cells produce and release the peptide hormone glucagon which acts to increase blood glucose levels through mechanisms of glycogenolysis (i.e., the breakdown of glycogen) and gluconeogenesis (i.e., the production of glycogen). Beta cells produce and release the peptide hormone insulin, which has the opposite effect of glucagon, in that insulin decreases blood glucose levels by aiding in glucose uptake into cells and decreases gluconeogenesis and glycogenolysis. Pancreatic beta cell destruction is the main cause of insulin deficiency, thus leading to IDDM. In these patients insulin injections, usually delivered via an insulin pump, are required to maintain blood glucose levels as well as other physiological functions. ¹⁰

As like most autoimmune diseases, the cause of IDDM is not fully understood. However, genetics, as well as the experienced environment, are the most characterized factors contributing to IDDM. ¹

1.1.3 Type 2 Diabetes Mellitus

Once commonly known as insulin-independent diabetes, type 2 diabetes mellitus (T2DM) is now better known as Insulin Resistant Diabetes. Regardless of its name, T2DM is the most prevalent form of diabetes and accounts for over 90% of diabetic cases.⁶ As with other types of diabetes, the development of T2DM is linked to both environmental and genetic factors.¹³ However, the risk factors for T2DM can be more readily controlled. Diet and obesity

are two of the most influential risk factors leading to this metabolic disease, and can be personally altered to reduce the associated consequences. Certain lifestyle changes, like diet and exercise, can fully improve the condition of patients with T2DM. ¹³

1.2 Gastrointestinal Tract Physiology

After nutrition (including glucose) is orally consumed, masticated and partial oral digestion has taken place, the food bolus is swallowed down the pharynx and travels through the esophagus by the process of peristalsis. Peristalsis is a wave of muscular contraction and relaxation that propagates the food bolus through the two esophageal sphincters into the cardiac stomach. The stomach and its contents then continue digestion by mixing the food bolus with gastric digestive juices containing HCl, digestive enzymes, mucus, etc. further breaking down the food bolus. Now called chyme, this mixture of digested food and the gastric juice cocktail are intermittently passed through the pyloric sphincter into the beginning of the small intestine known as the duodenum.

Upon chyme entering the small intestine, enteroendocrine cells of the duodenum release the peptide hormone cholecystokinin (CCK), which causes contraction of the gallbladder, releasing the liver produced bile into the duodenum. CCK also causes stimulation of the pancreas, releasing digestive enzymes into the intestine. The small intestine (duodenum, jejunum, & ileum) then continues mixing and digesting nutrients until they are absorbed through the intestine into the bloodstream. The waste that is not digested or absorbed into the bloodstream enters the large intestine (colon), where it is formed into fecal matter for expulsion.

While the fecal matter is awaiting expulsion from the rectum, the colon continues to reabsorb water.

1.3 Physiological Role of Bile Acids in Relation to Diabetes

1.3.1 What are Bile Acids?

Bile acids are primarily known for their roles in digestion, specifically in lipid and vitamin digestion and absorption.³ However, several research studies have reported the true importance of bile acids in relation to glucose homeostasis.²² Bile acids are amphipathic molecules, derived and synthesized from a steroid backbone of cholesterol, and are important for many physiological functions including facilitation of digestion.³ These cholesterol catabolites, containing both hydrophobic and hydrophilic regions, account for a major portion of the total daily turnover of cholesterol in humans and can act as strong detergents.⁸

The two categories of bile acids are primary bile acids - those synthesized in the liver, and secondary bile acids – those synthesized by symbiotic bacteria in the GI tract.

Chenodeoxycholic acid (CDCA) and cholic acid (CA) are the major primary bile acids, while deoxycholic acid (DCA) and lithocholic acid (LCA) are the major secondary bile acids in humans.^{7, 8}

1.3.1 Bile Acid Synthesis

In humans, most bile acids are conjugated (with glycine or taurine residues) and become bile salts, as they contain sodium. Becoming a conjugated bile salt (rather than a bile acid) increases the solubility, prevents Ca^{2+} build up, gives bile acid a negative charge, increases ionization, and helps prevent from enzymatic cleavage at physiological pH.^{8, 21} Most human bile acids are C_{24} -5 β -bile acids as they contain the 5 β hydrogen, 3 α hydroxyl group, and are in the cis conformation along the A and B rings. These bile acids have one 5-carbon ring fused among three 6-carbon rings.^{12, 24}

Two pathways exist for the synthesis of primary bile acids in the liver, the classic neutral pathway and the acidic alternative pathway.^{20, 22} The acidic pathway only accounts for a small portion of all primary bile acid synthesis (≥ 10 percent), leaving the neutral pathway to account for the vast majority of synthesis (≤ 90 percent).¹⁷ In the classic pathway, bile acids are derived from cholesterol by a process of reactions including hydroxylation, double bond saturation (C5=C6), epimerization, and oxidative cleavage.^{8, 20} The overall multistep process includes about seventeen different enzymes located throughout different organelles in a cell, including both the mitochondria and cytosol. Not only does the CYP7A1 (cholesterol 7 α hydroxylase) initiates primary bile acid synthesis, but it also controls the only rate limiting step.^{8, 17} Another enzyme, CYP8B1 (12 α hydroxylase), is responsible for determining which primary bile acid will be synthesized. In the presence of CYP8B1, cholic acid will be synthesized. In the absence of CYP8B1, chenodeoxycholic acid will be synthesized.^{12, 30}

In humans, an average of ~0.3 grams of bile acids are synthesized in the liver and excreted from the body daily (~0.5 g bile acids lost in fecal matter). *De novo* synthesis in the liver replaces bile acids lost as fecal matter. As primary bile acids are synthesized from

cholesterol in the liver, secondary bile acids are derived from primary bile acids in the gut.^{8, 17} Intestinal microbiota's enzymatic interaction converts primary bile acids into secondary bile acids. After conjugations are removed from primary bile acids, microbial 7 α dehydroxylase completes the conversion to secondary bile acids.²⁰ Cholic acid is converted to deoxycholic acid, and chenodeoxycholic acid is converted to lithocholic acid.¹⁷ Secondary bile acids are then either excreted as fecal matter or recirculated for further use. Most recirculated bile acids are quickly modified by sulfurylation conjugation in order to detoxify from the hydrophobic nature of bile acids. Soon after, these recirculated secondary bile acids are excreted into the bile.^{12, 20}

1.3.2 Bile Acid Circulation

After primary bile acids are made in the liver, they are secreted into the bile. Bile is then further concentrated and stored in the gallbladder, where it will remain until it is secreted through the bile duct into the small intestine.^{12, 21} After a meal, CCK is released from the intestinal enteroendocrine cells, specifically from the duodenum. CCK acts to release digestive enzymes from the pancreas and to cause contraction of the gallbladder releasing stored bile.²¹ The majority of bile acids in the small intestine are reabsorbed by enterocytes with microvilli in the ileum.^{8, 17} Bile acids then transport across enterocytes into the portal circulation where they are returned to the liver. Small amounts of bile acids are reabsorbed by the duodenum, jejunum, and the large intestine where they also return to the liver to be secreted into bile once again.¹² Other modes of bile acid transport include the cholangiohepatic shunt in which epithelial cells in the bile duct take up bile acids and take them back to the liver, and reabsorption by the kidneys after traveling through the systemic circulation.^{8, 20}

1.4 Physiological Effects of Glucagon-like Peptide 1 (GLP-1)

1.4.1 Real World Relevance and Overview

In recent years, there has been an increasing interest in coming up with new anti-diabetic therapies that can compete for treatment success. Among these innovative anti-diabetic topics of research, Glucagon-like Peptide 1 (GLP-1) has become an attractive and highly studied contender.² GLP-1, an enteroendocrine hormone also known as GCG, has become extremely attractive target for type 2 diabetes and obesity research due to its actions in improving glucose tolerance in mice.²⁵ Current treatments for T2DM and obesity involving GLP-1 include using GLP-1 receptor agonists and dipeptidyl peptidase 4 inhibitors, both of which will augment the effects of GLP-1. Hyperglycemia will be better controlled, and wanted weight loss will be promoted.^{2,5}

As previously stated above, GLP-1 is an enteroendocrine hormone/ neuropeptide secreted from specialized intestinal L-cells.⁵ These specialized epithelial cells are primarily located in the distal small intestine and colon, but can also be found dispersed throughout the entire intestine.²⁵ L-cells act as gut lumen nutrient sensors through mechanisms such as nuclear receptors, ion channels, and G protein-coupled receptors. Proteins, lipids, as well as carbohydrates, are nutrients sensed by L-cells in the intestinal tract that lead to the release of GLP-1. However, the release and action of GLP-1 are quickly ended due to its rapid enzymatic degradation by dipeptidyl peptidase 4 (DPP4). GLP-1 has the half-life of less than 2 minutes.²

1.4.2 Physiology of GLP-1

Glucagon-like Peptide 1 serves many diverse functions physiologically, having both peripheral as well as central actions. The main action of GLP-1 is through the incretin hormone effect, which causes postprandial insulin secretion from β -cells in the pancreatic Islets of Langerhans.¹¹ Not only does GLP-1 increase insulin release, but it also inhibits glucagon secretion. With insulin secretion increased and glucagon secretion decreased, glucose is taken up by cells (muscle, liver, adipose tissue) for storage or use. Glucose production will also be decreased, indicating that insulin sensitivity may be increased.³⁰ Other relevant actions of GLP-1 include the inhibition/decrease in gastric motility and food intake, thereby effecting glucose metabolism indirectly. While the full spectrum of GLP-1's effects are still being elucidated, GLP-1 increases pancreatic β cell proliferation, decreases pancreatic β cell apoptosis, increases protection and proliferation of neurons in the brain, and increases protection of the circulatory system.¹¹

1.4.2 Stimulated Secretion of GLP-1

The stimulation of GLP-1 release comes when organic meals containing carbohydrates, proteins or fat are consumed. Although there is not a fully accepted mechanism for the secretion of GLP-1, there are consistencies in the proposed mechanisms. Once organic matter is ingested, digested, and present in the intestinal lumen, it is thought that the sensation, absorption and metabolism of nutrients (especially glucose) causes the release of GLP-1 from L-cells.¹¹ Long and short chain fatty acids, as well as taste receptor stimulation, have also been shown to regulate

GLP-1 release. More importantly, bile acids are involved in the regulation of the stimulated secretion of GLP-1 when released from the gall bladder into the intestinal lumen.²⁵

1.5 Takeda G Protein-coupled Receptor 5 (TGR5) Functional Physiology

1.5.1 G Protein Coupled Receptors

GPCR are proteins containing seven transmembrane spanning regions, situated in cell membranes that are highly involved in many cellular response pathways and signal transduction.¹⁴ GPCRs are bound and coupled to three additional subunits α , β and γ . When extracellular ligands such as bile acids bind to these cell membrane receptors the extracellular signal is transduced through an intracellular signaling cascade involving many downstream effectors.²⁶ Specifically, when the GPCR becomes activated, a conformational change is induced, and GDP is exchanged for GTP (GEF-like function). Next, the complex disassociates into dimers. β - γ breaks away from G- α , and G- α then activates the enzyme adenylyl cyclase (AC). AC activation gives rise to cAMP and protein kinase A (PKA), which then induce additional signaling downstream. It is also important to note that GPCRs are in a low affinity state during resting conditions.¹⁴

There are over eight hundred characterized G-protein Coupled Receptors (GPCRs). However, only a select few of these GPCRs are known to have the functional capability of being regulated and modulated by bile acids. A major subgroup of GPCRs, rhodopsin-like receptor class (Class A), are regulated by bile acids.¹⁴ GPCRs are continuing to be studied as they are attractive targets for therapeutic agonists and treatment for many diseases and syndromes.^{26, 31}

1.5.2 Overview and Location

Not only are bile acids involved in digestion, but they also serve an important physiological role in their receptor activations. Among these bile acid mediated receptors is the G protein-coupled receptor (GPCR), Takeda G Protein-coupled Receptor 5 (TGR5). TGR5, also known as G protein-coupled bile acid receptor 1 (GPBAR1), is a membrane bound receptor that is stimulated by both primary and secondary bile acids.¹⁴ Ubiquitously located, TGR5 is most densely expressed in areas such as brown adipose tissue, placenta, gallbladder, and intestine (especially in L-cells). Other tissues contain a varying degree of TGR expression including enteric neurons, hepatocytes in the liver, skeletal muscle as well as macrophages and monocytes.²⁷

1.5.2 Physiology of TGR5

The physiological role of TGR5 in relation to diabetes, as well as other physiological processes and diseases, is not completely understood. However, various physiological functions of TGR5 have been found and studied since its discovery in 2002.²⁶ Not only has it been shown that activation of the TGR5 receptor increases energy expenditure in various tissues such as brown adipose tissue and skeletal muscle, but also TGR5 modulates immune responses and processes such as inflammation.⁷ More importantly, TGR5 activation has multiple effects in bile acid homeostasis and glucose metabolism.¹⁵

Multiple bile acids have been shown to activate the TGR5 receptor in both mouse and human tissues. The conjugated and unconjugated forms of LCA, DCA, CDCA, and CA all have been shown to activate TGR5, with LCA and DCA having the highest natural potencies. It is important to note that the TGR5 receptor has essentially the same affinity for natural bile acids in both mouse and human tissues. However, synthetic agonists of mouse and human TGR5 show a varying degree of affinity.^{7, 31}

1.5.4 TGR5 and Glucose Metabolism

As previously mentioned, TGR5 is highly expressed in intestinal L-cells.¹⁸ Once this plasma membrane receptor is activated by ligands (such as bile acids released from the gall bladder in response to meal consumption), the release of GLP-1 is promoted from L-cells in the intestine.¹⁵ The exact mechanism of TGR5 stimulated GLP-1 release is not fully understood. However, it is believed that the increased intracellular cAMP from the TGR5 GPCR activation mechanism is directly involved with intestinal secretion of GLP-1. Also, calcium is needed for the release of GLP-1.

Earlier studies have shown that GLP-1 secretion is regulated by TGR5 activation in cultured mouse STC-1 cells. It has also been reported that the synthetic bile acid INT-777, also known as 6-ethyl-23(S) methyl-cholic acid (6EMCA), is a potent TGR5 agonist that induces GLP-1 release in mouse intestinal STC-1 cells and human intestinal NCI-H716 cells.^{4, 29} Furthermore, it has been demonstrated that TGR5 silencing by shRNA prevented GLP-1 release from STC-1 cells. Insulin resistant and obese mice with a gain of function of TGR5 have

demonstrated healthier pancreatic islets of Langerhans, and thus an increased glucose tolerance due to the increase in GLP-1 release. In other studies, activation of TGR5 in macrophages found in adipose tissue improve the actions of insulin. Another study demonstrated agonist TGR5 activation decreased hemoglobin A1C levels, and even decreased fasting plasma glucose levels in mice with T2DM. These TGR5 mediated improvements in glucose homeostasis provide insight for the development of new treatments for metabolic diseases such as T2DM and obesity. Perhaps, TGR5 agonists can be used as potential drugs for targeted therapy. 18

1.6 Bile Acid Induced Farnesoid X Receptor (FXR) Regulation

1.6.1 Overview and Location

Not only do bile acids act through membrane receptors on the cell surface, but they also have actions in activating nuclear receptors. Of these nuclear bile acid receptors, Farnesoid X Receptor (FXR) seems to be one of the most important.⁴ FXR expression has been detected in numerous tissues, with the highest expression density being found in the adrenal gland, kidneys, liver, small intestine, and colon.³⁴ FXR, also known as NR1H4, serves a wide variety of physiological roles and more research is needed to determine the validity of the findings.¹⁶

1.6.2 Physiological Role of FXR

Numerous studies have shown that FXR play crucial roles in bile acid homeostasis and cholesterol metabolism.²⁰ Specifically, this transcription factor regulates the recycling and

circulation of bile acids between the intestine and liver, as well as controls the biosynthesis of bile acids through negative feedback regulation. FXR also inhibits other receptors and nuclear transcription factors in order to regulate the repression of bile acid biosynthesis.¹⁹

The FXR receptor has been shown to be activated by many natural and synthetic ligands, each of which can have various physiological outcomes.²⁹ Primary bile acids seem to bind to FXR with a greater affinity, and the relative affinities for FXR are CDCA>DCA=LCA>CA.³² In contrast, the bile acid sequestrates (BAS) complex has been shown to decrease FXR activation in the intestines.³²

1.6.3 FXR and Glucose Metabolism

Among the understood actions of bile acids involved in digestion and absorption of lipids and vitamins, bile acids seem to be acting as metabolic regulators as well.³³ It has been shown that FXR activation lowers blood glucose levels in both wild type and db/db mice. Demonstrated by the same study was that FXR activation in db/db mice enhanced insulin sensitivity by increasing glycogen synthesis and thereby repressing hepatic gluconeogenic genes.³⁴ Another study showed that FXR actually reversed insulin resistance and corrected lipid abnormalities.^{9, 29}

However, due to the high degree of variability in bile acid actions, some studies reported agonist FXR activation led to the induction of diabetes and obesity. FXR may not prove useful for long term treatment of such metabolic syndromes and diseases, as it decreases bile acid concentrations and increases insulin resistance leading to weight gain.³³ Furthermore, FXR activation has been shown to inhibit GLP-1 production in L cells by actions of decreasing

proglucagon expression.¹⁶ When FXR is deficient or inhibited proglucagon and GLP-1 expression increases. FXR along with TGR5 and GLP-1 will continued to be studied as potential targets for metabolic treatment therapy, specifically in relation to diabetes mellitus.^{19, 28}

Hypothesis

It was hypothesized that expression of GLP-1 is negatively modulated by bile acid nuclear receptors (FXR) and positively modulated by bile acid GPCRs (TGR5), and that a decrease in FXR expression and increase in TGR5 expression in diabetes leads to an increase in GLP-1 expression and release.

2. MATERIALS AND METHODS

2.1 Reagents

High and low glucose Dulbecco's Modified Eagle Medium (DMEM), as well as 0.25% Trypsin-EDTA (1X) and antibiotics (Penicillin-Streptomycin, & Gentamycin Sulfate), used for STC-1 cell cultures were obtained from ThermoFisher Scientific. Fetal Bovine Serum (FBS), Amphotericin B were also provided by Fisher Scientific. Trizol Tissue RNA Extraction reagents were obtained from Invitrogen. RNAqueous-Micro Total RNA Isolation Kit used for STC-1 cells was provided by Ambion. TaqMan mRNA Reverse Transcription Kit was obtained from Applied Biosystems. TGR5 (GPBAR1), FXR (NR1H4), GLP-1 (GCG), and GAPDH PCR primers and assay mixes were obtained from ThermoFisher Scientific. TaqMan Universal qPCR Master Mix containing AmpliTaq Gold DNA polymerase, and TGR5/FXR/GLP-1/GAPDH assay mixes with primers and probe were all obtained from Applied Biosystems.

2.2 Animals

Wild-Type mice (C57BL/6) and db/db mice (BKS.Cg-DOCK 7m+Leprdb/db) were purchased from Jackson Laboratories. Mice had access to food and water ad libitum, housed in a light/dark cycle (12h/12h), and euthanized by asphyxiation with carbon dioxide; as approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University. The animals were housed in the animal facility administered by the Division of Animal Resources, at Virginia Commonwealth University. All procedures were conducted in accordance with the Institutional Animal Care and Use Committee of the Virginia Commonwealth University.

2.3 Tissue Preparation

After mice were sacrificed by carbon dioxide (CO₂) asphyxiation, specimen blood glucose levels and weights were measured. The body weight average was 29.7 grams for Wild-type mice and 144.9 grams for db/db mice. Furthermore, the blood glucose level average was 155.6 mg/dL for Wild-type mice and 526 mg/dL for db/db mice. Whole organs, both small intestine and colon, were then rapidly removed. The contents (digested material) inside the small and large intestine were emptied, and hollow whole organs were placed in 1X PBS. The mucosa was then scraped from these isolated organs and collected in micro centrifuge tubes for RNA Isolation.

2.4 Cell Culture

2.4.1 Medium Preparation

Dulbecco's Modified Eagle Medium (DMEM-10 containing either 4.5g/L D-Glucose or 1g/L D-Glucose, 110 mg/L sodium pyruvate and L-glutamine) was prepared with the addition of filtered 10% Fetal Bovine Serum (FBS), Penicillin (200 U/ml), Streptomycin (200 ug/ml), Gentamycin sulfate (50 mg/ml), and unfiltered amphotericin B (2.5 ug/ml).

Low glucose (5.5 mM) DMEM-0, high glucose (25mM) DMEM-0, and super high glucose (40mM) DMEM-0 were also prepared without the addition of antibiotics, Fetal Bovine

Serum or amphotericin B. High and Low Glucose DMEM-0 was used for experimental conditions, while DMEM-10 was used for STC-1 cell culture maintenance.

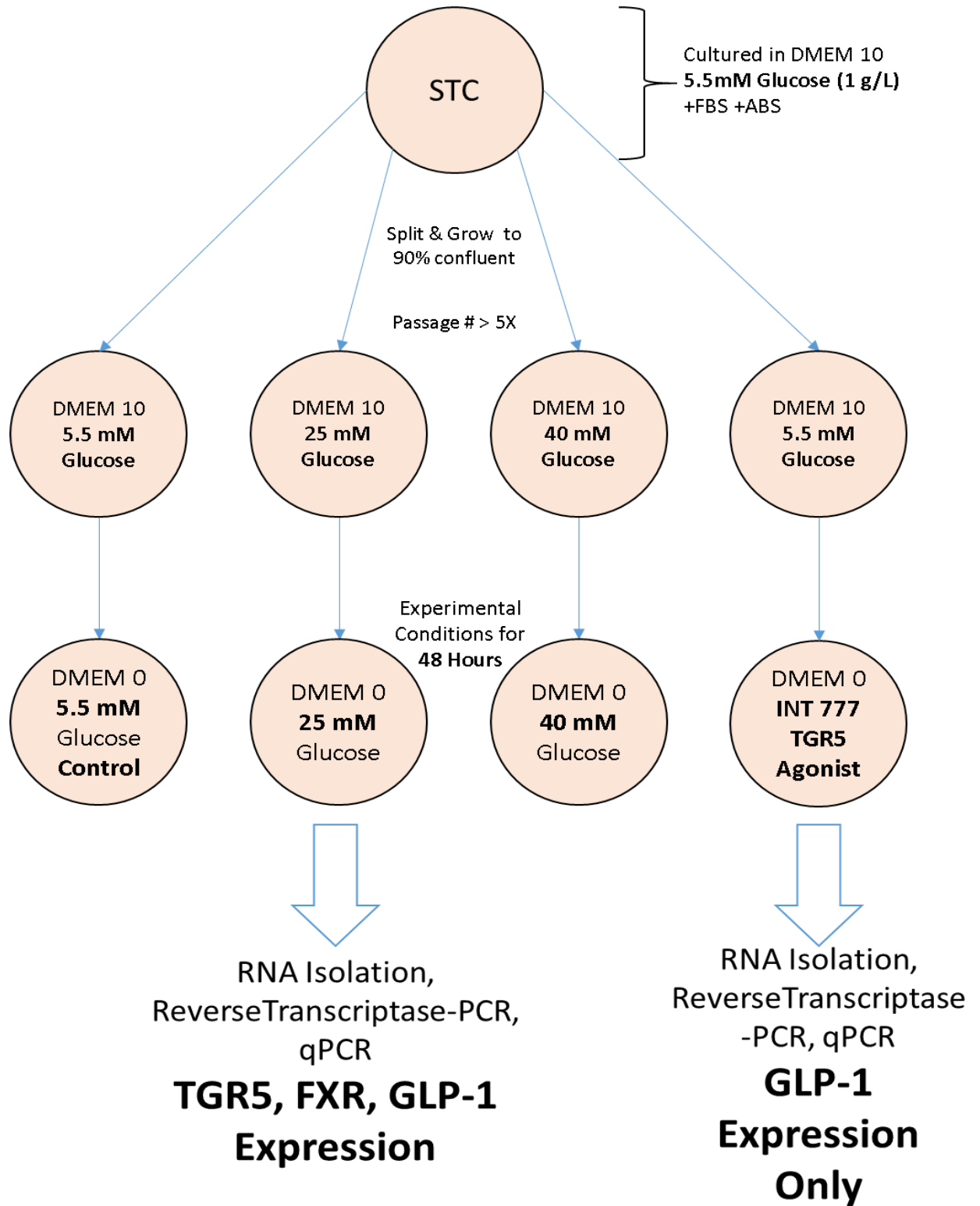
2.4.2 Experimental Cell Culture Conditions

The Secretin Tumor Cell Line (STC-1), derived from murine enteroendocrine tumors, was used for experimental cell culture research in vitro. Two separate method sets of growing conditions were used to test TGR5, FXR, and GLP-1 mRNA expression level changes. A visual representation of the growing/experimental conditions is provided below (Figure 1).

Figure 1: STC-1 Cell Line Experimental Method.

In this cell culture method, cells were grown in DMEM-10 containing either 5.5mM, 25mM or 40mM glucose and incubated in a 37°C, 10% CO₂ humidified atmosphere. Cell cultures were passaged at 90% confluent, and passages 5-10 were used for experimentation. Once grown to 90% confluency after enough passages, cell culture experimental conditions were set up. For 48 hours, cells were cultured in DMEM-0 containing either 5.5mM, 25mM, or 40mM glucose, as well as a separate culture in 5.5mM glucose DMEM-0 with the addition of the TGR5 agonist, INT-777 (30 μM). INT-777 was sonicated separately using the Fisher Scientific™ Model 705 Sonic Dismembrator before its addition to cell cultures. After 48 hour experimentation, TGR5, FXR, and GLP-1 mRNA expression levels were quantified.

Figure 1:
Cell Culture Method



2.5 RNA Isolation

2.5.1 Tissue RNA Isolation

Total RNA was extracted and isolated from mouse tissue colon and intestine mucosa using the Trizol extraction method. Mouse intestine and colon mucosal cells were first re-suspended with Trizol provided by Invitrogen. After a 5 minute incubation at room temperature, chloroform was then added to separate out unwanted cell components. Centrifugation at 12000g for 15 minutes at 4°C was used to assist in the separation of the organic and aqueous phase. The chloroform separation was completed and repeated an additional 2 times to ensure adequate separation of cell components. Next, 2-propanol (isopropyl alcohol) was added to the retained aqueous phase, and the mixture was incubated at -80°C overnight. The next day, the mixture was separated by centrifugation (12000g x 15 mins x 4°C). The supernatant was discarded, and ice cold 75% ethanol was added to wash the RNA pellet. The final RNA pellet was then collected by centrifugation and allowed to air dry for 10 minutes.

RNA concentrations and purities were obtained by dissolving the RNA pellet in Ultrapure™ DEPC H₂O, making a 50X dilution, and measured in a Beckman Coulter DU 530 UV/Vis Spectrophotometer.

2.5.2 Cell RNA Isolation

RNA was extracted and isolated from STC-1 cells using the Ambion™ RNAqueous-Micro Total RNA Isolation Kit. STC-1 cultured cells were first detached from 10 cm plates using

0.25% Trypsin-EDTA (1X), and then collected/ pelleted through centrifugation at 2000 rpms for 5 minutes at room temperature. Cells were re-suspended and washed with 1X Phosphate-buffered saline (PBS) and then lysed using a lysis buffer containing guanidinium thiocyanate, which quickly inactivates ribonuclease and disrupts cell membranes.

After being vigorously vortexed, 64% ethanol was then added and mixed into the ice cold lysate. The mixture was transferred to RNA columns containing a silica filter, and centrifuged at 14000 rpms for 1 min at 4°C. The solution was discarded and the filter was washed with Wash Solution 1 and centrifuged with the previous listed conditions. Again, the solution was discarded and then washed two additional times with Wash Solution 2/3. The RNA column was then placed in a new autoclaved centrifuge tube. The highly concentrated RNA was eluted using preheated 95°C Elution Solution, and collected through centrifugation at 14000 rpms for 1 min at 4°C.

An occasional DNase treatment and DNase inactivation were used to remove any suspected DNA contamination. RNA purities, as well as concentrations were then obtained using a ThermoScientific NanoDrop 8000 UV-Vis Spectrophotometer.

2.6 Reverse Transcription PCR (RT-PCR)

Total isolated RNA was converted to cDNA using the TaqMan mRNA Reverse Transcription Kit prepared by Applied Biosystems. A master mix was prepared containing 25X dNTP mix (100mM), 10X RT Buffer, 10X RT Random Primers, RNase Inhibitor (20 U/ul), MultiScribe Reverse Transcriptase (50 U/ul), and nuclease free water. Isolated RNA samples

were combined with RT master mix and placed in a thermocycler under the following optimized conditions: 1) 25°C for 10 minutes, 2) 37°C for 120 minutes, 3) 85°C for 5 minutes, 4) 4°C infinite hold.

2.7 Conventional PCR

PCR was used to ensure the expression of target genes in related tissue. Optimal cycle number and annealing temperatures were obtained experimentally for each target and are as follows: TGR5 (35 cycles, 56°C), FXR (35 cycles, 56°C), GLP-1 (35 cycles, 59.5°C), and GAPDH (30 cycles, 55°C). PCR was run under the following thermocycler conditions: 95°C initial denaturation for 30 seconds, multiple cycles of 95°C - 30 seconds, 55°C – 60 seconds, and 68°C – 60 seconds/kb, followed by a final extension of 68°C for 5 minutes and an infinite hold at 4°C.

PCR primer sequences are as follows: Mm-TGR5-F = 5'-ACT GGT CCT GCC TCC TTC TC-3', Mm-TGR5-R = 5'-GAA GAC AGC TTG GGA GCT GC-3', Mm-FXR-F = 5'-GCG AAG GGC GTG ACT TGC GA-3', Mm-FXR-R = 5'-AGG AGG GTC TGT TGG TCT GCC G-3', Mm-GLP1-F = 5'-TCA TCC CCA GCT TCC CAG ACA-3', Mm-GLP1-R = 5'-TCT GGG AAG TCT CGC CTT CCT-3', Mm-GAPDH-F = 5'-AGA AAC CTG CCA AGT ATG ATG-3', Mm-GAPDH-R = 5'-GGA GTT GCT GTT GAA GTC G-3'.

2.8 Real-Time PCR (qPCR)

Singleplex Quantitative Polymerase Chain Reaction was performed on RT-PCR product (cDNA) synthesized from extracted RNA from STC-1 cell cultures and mouse (WT and db/db) mucosa samples (whole small intestine and colon). The Applied Biosystems StepOne™ Real-Time PCR System was used at optimized gradient thermocycler conditions. qPCR thermocycler conditions were: 40 cycles of 50°C for 2 minutes, 95°C for 10 minutes, 95°C for 15 seconds, and 60°C for 1 minute.

TaqMan Reagents, including universal master mix and primer/probe assay mixes, were used for each cDNA sample and combined into a 20 ul reaction volume. Applied Biosystems TaqMan Universal PCR Master Mix (2X) contained Uracil-DNA Glycosylase, AmpliTaq Gold DNA Polymerase, dNTPs, dUTPs, and other buffer components for optimized performance. The assay mixes used for real-time PCR are as follows: Glp-1/Gcg = Mm00801714_m1 (85 amplicon length), TGR5/Gpbar1 = Mm04212121_s1 (104 amplicon length), FXR/Nr1h4 = Mm01240550_m1 (110 amplicon length), GAPDH = Mm99999915_g1 (107 amplicon length).

2.9 Gene Expression Quantification

Relative quantification and absolute quantification can be used as quantification methods for gene expression quantification. In using absolute quantification, one can use either the digital PCR method or the standard curve method. The absolute quantification digital PCR method requires no standards, and is solely based on quantifying the target of interest by the number of digital PCR replicates. The absolute quantification standard curve method relies on quantifying

targets to know standards. Usually, a standard curve is created first and used for comparison to the unknown target quantity.

In relative quantification, target gene expression levels are analyzed by comparing to an untreated reference sample (control housekeeping gene). Quantification is then calculated using delta delta CT values to obtain fold changes in expression. CT, also known as CP, refers to the cycle threshold or the number of cycles it takes for the florescence to reach and cross its threshold. Florescent thresholds were calculated using the StepOne™ RT-PCR System. Many other methods exist for interpreting the quantification values including the Delta Delta CT Method ($\Delta\Delta CT$) shown below:

$$\Delta\Delta CT = (C_{T, Tar} - C_{T, HKG})_{Treated} - (C_{T, Tar} - C_{T, HKG})_{Control}$$

$$R = 2^{-\Delta\Delta CT}$$

Tar is the target gene being evaluated, HKG refers to the housekeeping gene, and R represents the fold change. For example, GAPDH was chosen as the housekeeping gene used in this study because it is reliable and expressed in all cells. It is important to note that the housekeeping gene must not be altered or changed by the experimental treatment, and is necessary to normalize the gene expression levels of the target.

2.10 Agarose Gel Electrophoresis (AGE)

Amplified PCR products were further analyzed and confirmed using Agarose Gel Electrophoresis. 1.5 % agarose gels were used containing 0.1 ug/ml Ethidium Bromide. 1X TAE buffer was used as the electrophoresis buffer. Gels were run under a constant voltage of 110 volts using the BioRad Powerpac 200, and then analyzed using the BioRad Gel Doc EZ Imager with Image Lab software.

2.11 Statistical Analysis

Data and results are presented as means \pm Standard Error Mean (SEM). All experiments were completed three or more times. Expression of GLP-1, TGR5 and FXR data in tissue and cells were analyzed for statistical significance using unpaired student T tests. GLP-1 expression in cells was also analyzed using a one-way ANOVA followed by Bonferroni *post hoc* tests. *p* values < 0.05 were considered significant.

3. RESULTS

3.1 Expression of TGR5, FXR, & GLP-1 in Diabetic Mouse Intestine and Colon Mucosa

The presence of TGR5, FXR and GLP-1 mRNA expression was first detected in both wildtype and diabetic (db/db, BKS.Cg-DOCK 7m+Leprdb/db) whole mouse intestine (small and large intestines) mucosa using agarose gel visualization of PCR products (data not shown). To evaluate the expression levels of TGR5, FXR, and GLP-1, isolated mouse whole small intestine, colon mucosa, and whole colon mucosa were quantified using Real-time PCR.

TGR5 mRNA expression is significantly increased ($p < 0.01$) in diabetic mouse tissue in both whole small and large intestine mucosa samples (Figure 2). A TGR5 mRNA fold change of 2 was noted in both small intestine and colon of db/db samples when compared to the wildtype control.

FXR mRNA expression is significantly decreased ($p < 0.01$) in diabetic mouse tissue in both whole small and large intestine mucosa samples (Figure 3). A FXR mRNA fold change of -0.6 and -0.5 was noted in small intestine and colon, respectively, in db/db samples when compared to the wildtype control.

GLP-1 mRNA expression is significantly increased in diabetic mouse tissue in both whole small intestine ($p < 0.05$) and whole large intestine mucosa samples ($p < 0.01$) (Figure 4). A GLP-1 mRNA fold change of 2 and 1.5 was noted in both small intestine and colon, respectively, in db/db samples when compared to the wildtype control.

3.2 TGR5, FXR, & GLP-1 Expression in Control and Hyperglycemic STC-1 Cells

The intestinal enteroendocrine cell line (STC-1) was examined for the presence of TGR5, FXR, GLP-1 expression, as well as expression of the housekeeping gene GAPDH. Expression detection was obtained from RNA isolated from STC-1 cells. PCR products of expected size were detected using target specific primers and agarose gel electrophoresis. GAPDH has 122 base pairs, TGR5 has 239 base pairs, FXR has 93 base pairs, and GLP-1 has 308 base pairs (Figure 5).

STC-1 cells were tested and cultured in hyperglycemic medium (25mM), and compared to cell cultures grown in normal glucose medium (5.5mM control). TGR5, FXR and GLP-1 expression was further analyzed and quantified using quantitative PCR (qPCR). Compared to the control, the hyperglycemic conditioned STC-1 cell line showed an increased quantification of TGR5 mRNA data ($p < 0.01$). Furthermore, a 2 fold increase in TGR5 mRNA expression was observed (Figure 6).

Additionally, FXR and GLP-1 mRNA quantification levels were significantly increased in STC-1 cells cultured and tested in hyperglycemic DMEM (25mM) for 48 hours (Figure 7; $p < 0.01$). FXR expression showed a fold change of 1.7, while GLP-1 expression showed a fold change of 2.6 when compared to the control.

3.3 Effect of Hyperglycemia in STC-1 Cells on GLP-1 Expression

The STC-1 cell line was further tested for the effect of different glucose concentrations, or degree of hyperglycemia, in the growing medium (DMEM) have on GLP-1 mRNA expression and quantification.

Three different glucose concentrations were used in the growing medium, and are as follows: 5.5mM of glucose was used as the control, as it is the normal concentration of glucose used for STC-1 cell line cultures; 25mM was used as a mild hyperglycemic condition, as it is also used for many STC-1 cell culturing conditions; and 40mM was used as the extreme hyperglycemic glucose concentration.

GLP-1 RNA expression and quantification levels were significantly increased in both STC-1 cells cultured with 5.5mM glucose DMEM (control) ($p < 0.01$) and 25mM mild hyperglycemic growing medium ($p < 0.01$) (Figure 9). More importantly, levels of GLP-1 expression in the extreme high condition treated with 40mM glucose DMEM were increased more than in cells treated with 25mM glucose DMEM ($p < 0.01$). Furthermore, GLP-1 quantification fold change was 2.6 for cells treated with 25mM glucose and 3.3 for cells treated with 40mM glucose, when compared to the control. It is also important to note that data for experiments treated with 25mM and 40mM glucose medium were only significant when compared to the control ($p < 0.01$), as opposed to the nonsignificant comparison between the two (25mM vs 40mM) ($p > 0.05$).

3.4 Effect of INT-777 Treatment on GLP-1 Expression in STC-1 Cell Line.

Intestinal enteroendocrine STC-1 cells, cultured in 5.5mM glucose DMEM, were then tested for the effect that homogenized INT-777 (a TGR5 receptor agonist) treatment (48 hours) would have on GLP-1 expression. GLP-1 mRNA expression and quantification levels were measured using quantitative-PCR. Quantification of GLP-1 was found to be significantly increased in STC-1 cells treated with INT-777 ($p < 0.01$). When compared to basal expression

levels (control), GLP-1 expression fold change in STC-1 treated with TGR5 agonist was shown to be 1.7. (Figure 10)

In Summary,

1. TGR5, FXR, and GLP-1 are expressed in wildtype and db/db whole mouse intestine mucosa and whole mouse colon mucosa.
2. TGR5 and GLP-1 mRNA expression levels are increased in diabetic mouse tissue (db/db), while FXR mRNA expression levels are decreased in diabetic mouse tissue (db/db).
3. TGR5, FXR, and GLP-1 are expressed in STC-1 cell line.
4. TGR5, FXR, and GLP-1 mRNA expression levels are increased in STC-1 cell line under hyperglycemic conditions.
5. GLP-1 quantification levels increased linearly with an increase in glucose concentration (5.5mM control, 25mM, and 40mM) in growing medium of STC-1 cells.
6. Expression levels of GLP-1 are increased in STC-1 cells treated with the TGR5 receptor agonist, INT-777.

Figure 2: Expression of TGR5 mRNA in Mouse Mucosa Tissue. TGR5 mRNA expression was identified and quantified from isolated (A) mouse whole small intestine mucosa and (B) mouse whole colon mucosa in both wild-type mice and db/db mice. TGR5 expression mRNA fold change is increased in db/db mice in both mouse whole small and large intestines. Values are represented by the means \pm SEM, (n = 3). * p < 0.05, **p < 0.01 vs WT.

Figure 2:

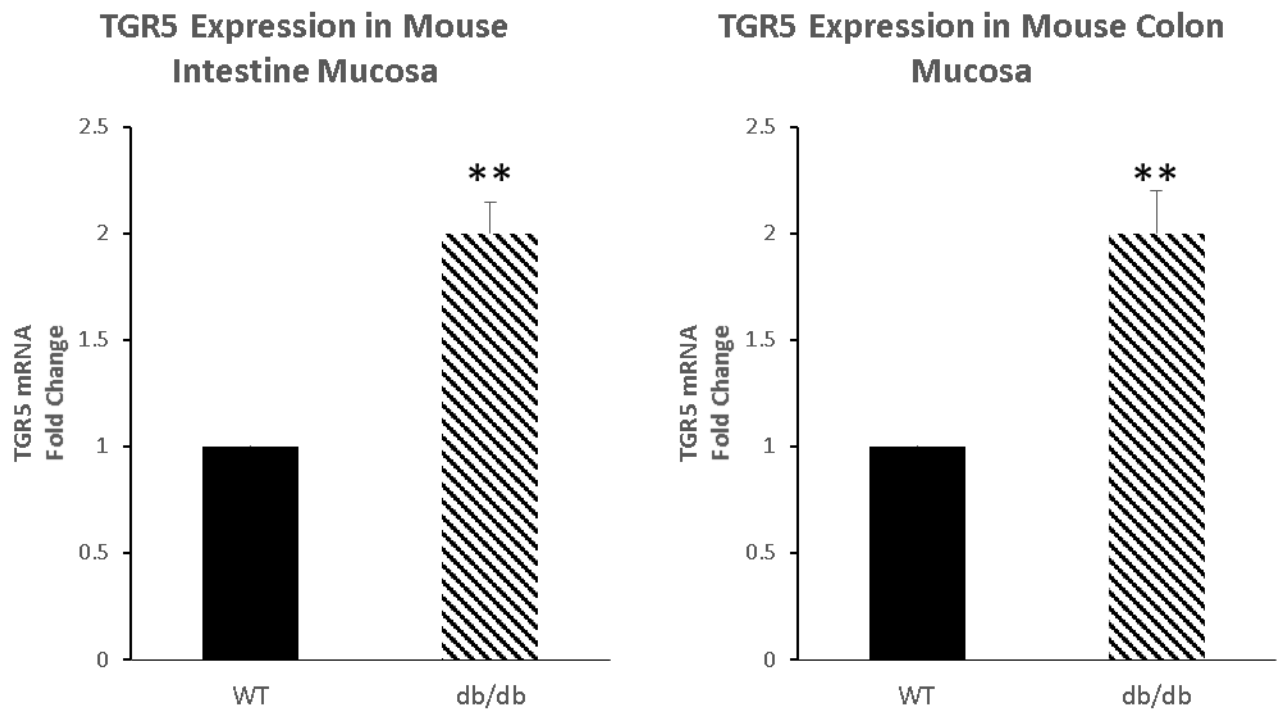


Figure 3: Expression of FXR mRNA in Mouse Mucosa Tissue. FXR mRNA expression was identified and quantified from isolated (A) mouse whole small intestine mucosa and (B) mouse whole colon mucosa in both wild-type mice and db/db mice. FXR expression mRNA fold change is decreased in db/db mice in both mouse whole small and large intestines. Values are represented by the means \pm SEM (n=4). * p < 0.05, **p < 0.01 vs WT.

Figure 3:

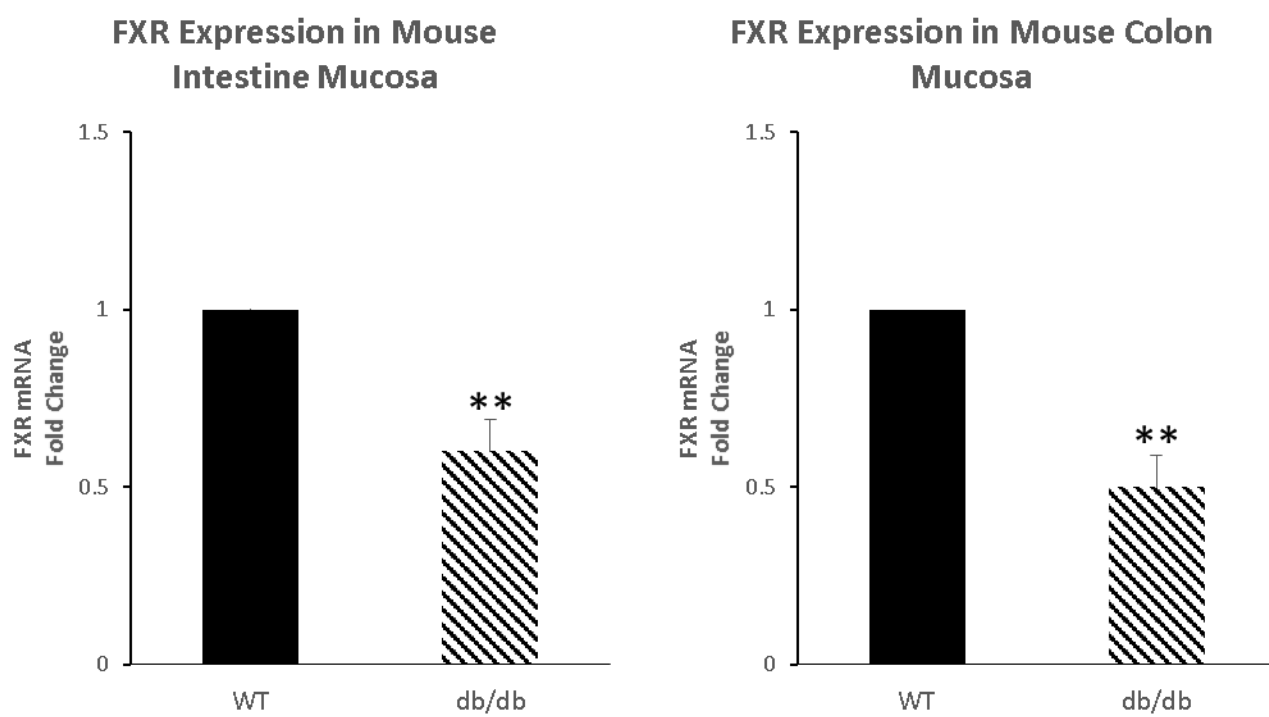


Figure 4: Expression of GLP-1 mRNA in Mouse Mucosa Tissue. GLP-1 mRNA expression was identified and quantified from isolated (A) mouse whole small intestine mucosa and (B) mouse whole colon mucosa in both wild-type mice and db/db mice (BKS.Cg-DOCK 7m+Leprdb/db). GLP-1 expression mRNA fold change is increased in db/db mice in both mouse whole small and large intestines. Values are represented by the means \pm SEM in 3 independent experiments (n=3). * p < 0.05, **p < 0.01 vs WT.

Figure 4:

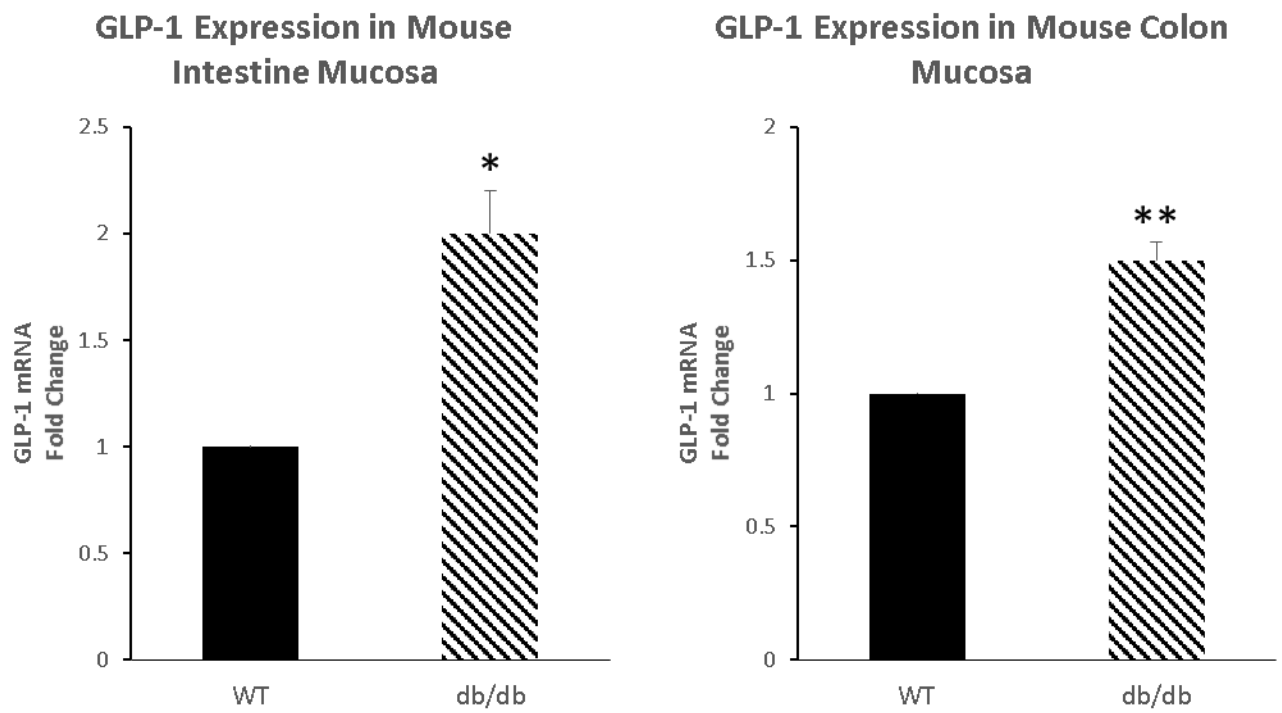


Figure 5: Detection of GAPDH, TGR5, FXR, and GLP-1 mRNA in STC-1 Cells. Target expression (GAPDH, TGR5, FXR, and GLP-1) was identified in cultured intestinal STC-1 cells by PCR. PCR products of expected size were detected using target specific primers and visualized on 1.5% agarose gels. GAPDH = 122 base pairs, TGR5 = 239 base pairs, FXR = 93 base pairs, and GLP-1 = 308 base pairs.

Figure 5:

mRNA Expression in STC-1 Cells

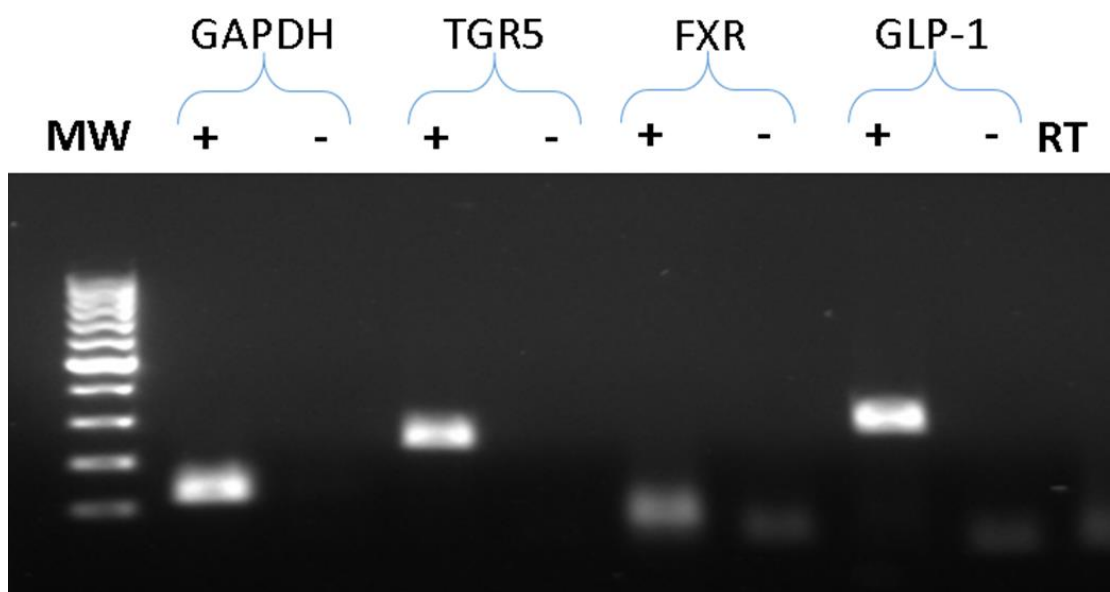


Figure 6: Analysis of TGR5 mRNA Expression in Hyperglycemic STC-1 Cell Line. TGR5 mRNA expression and quantification were measured using quantitative-PCR from the STC-1 cell line in both cell cultures grown in hyperglycemic glucose medium (25mM) and cells cultured in normal glucose medium (5.5mM control). When compared to the control, TGR5 mRNA quantification levels were observed to be higher in STC-1 cells cultured in hyperglycemic growing medium. Values are represented by the means \pm SEM in 4 independent experiments (n=4). * $p < 0.05$, ** $p < 0.01$ vs normal glucose control.

Figure 6:

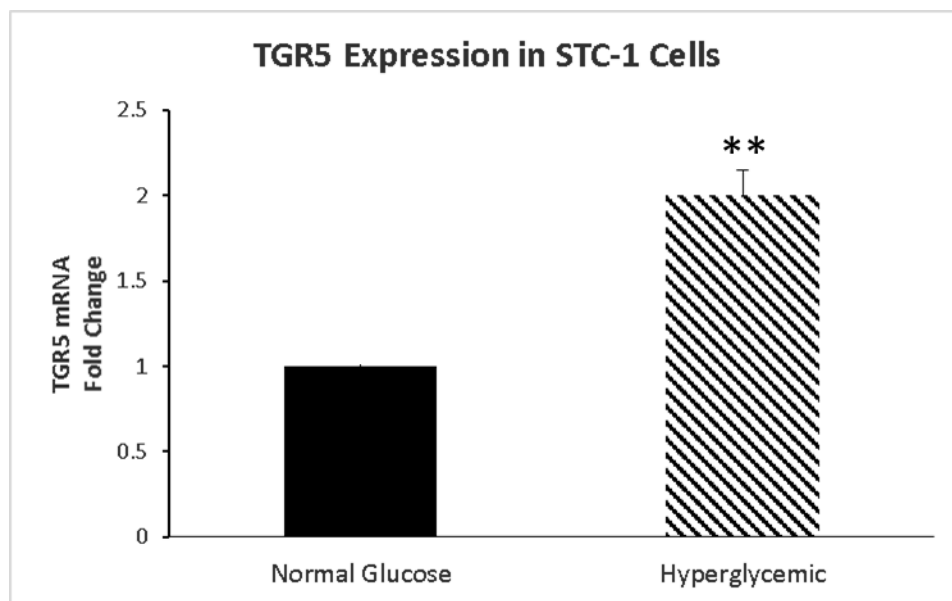


Figure 7: Analysis of FXR mRNA Expression in Hyperglycemic STC-1 Cell Line. FXR mRNA expression and quantification were measured using quantitative-PCR from the STC-1 cell line in both cell cultures grown in hyperglycemic glucose medium (25mM) and cells cultured in normal glucose medium (5.5mM control). When compared to the control, FXR mRNA quantification levels were observed to be higher in STC-1 cells cultured in hyperglycemic growing medium. Values are represented by the means \pm SEM in 4 independent experiments (n=4). * p < 0.05, **p < 0.01 vs normal glucose control.

Figure 7:

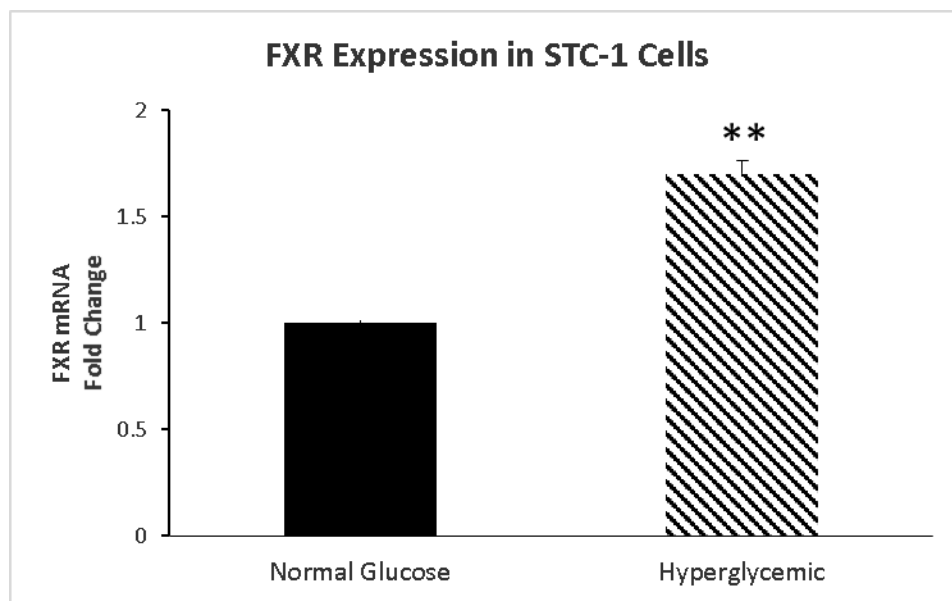


Figure 8: Analysis of GLP-1 mRNA Expression in Hyperglycemic STC-1 Cell Line. GLP-1 mRNA expression and quantification were measured using quantitative-PCR from the STC-1 cell line in both cell cultures grown in hyperglycemic glucose medium (25mM) and cells cultured in normal glucose medium (5.5mM control). When compared to the control, GLP-1 mRNA quantification levels were observed to be higher in STC-1 cells cultured in hyperglycemic growing medium. Values are represented by the means \pm SEM in 4 independent experiments (n=4). * p < 0.05, **p < 0.01 vs normal glucose control.

Figure 8:

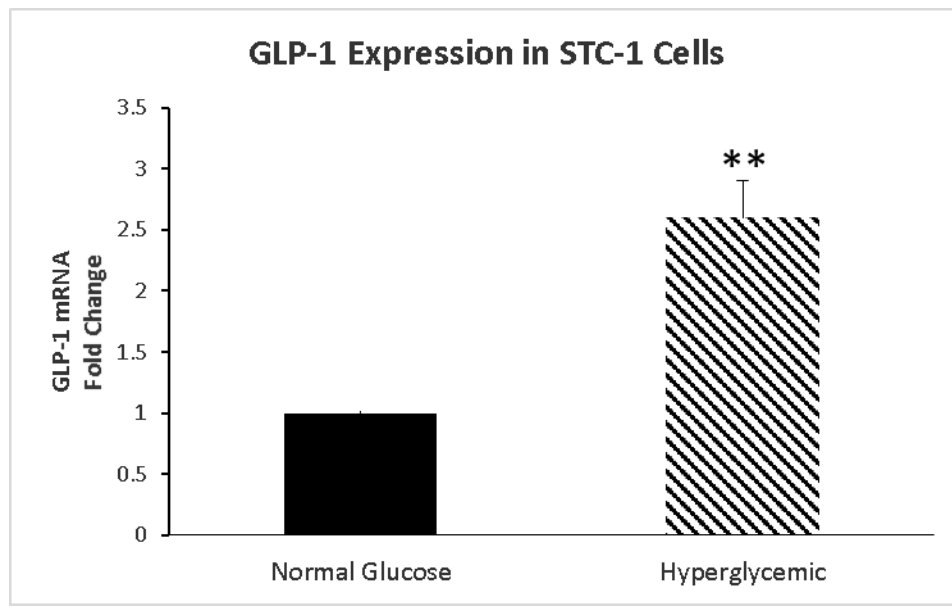


Figure 9: Effect of Hyperglycemia on GLP-1 mRNA Expression in STC-1 Cells.

GLP-1 mRNA expression and quantification were measured using quantitative-PCR from the STC-1 cell line in cells cultured in DMEM with varying glucose concentrations. 5.5mM (control), 25mM (hyperglycemic), and 40mM (extreme hyperglycemic) glucose concentrations were used. When compared to the control, an increase in GLP-1 RNA expression levels was shown in both STC-1 cells cultured in 25mM and 40mM glucose growing medium. Furthermore, quantification levels in cells cultured and tested in 40mM glucose DMEM were even higher than in cells with 25mM glucose DMEM. Values are represented by the means \pm SEM in 3 independent experiments (n=3). *p < 0.05, **p < 0.01 vs normal glucose control.

Figure 9:

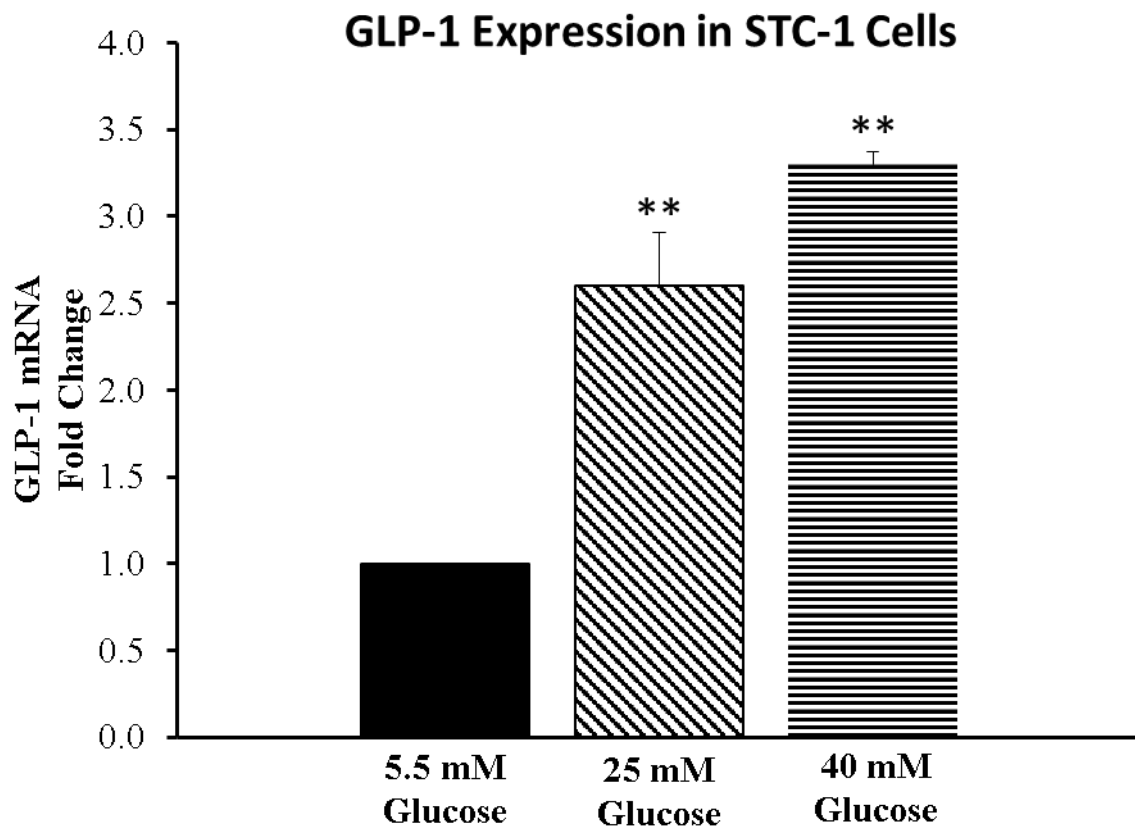
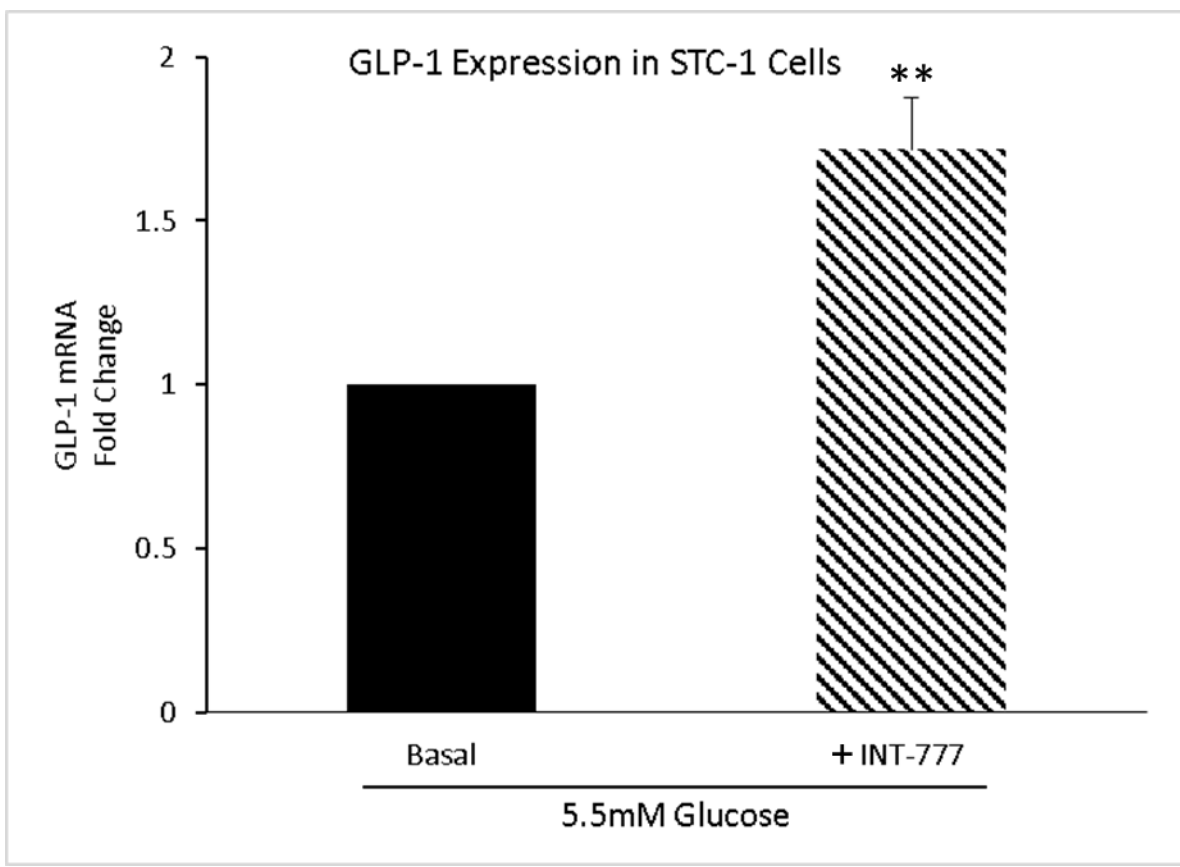


Figure 10: Effect of INT-777 Treatment on GLP-1 mRNA Expression in STC-1 Cells.

GLP-1 mRNA expression and quantification were measured using quantitative-PCR from the STC-1 cell line in cell cultures grown in normal DMEM (5.5mM glucose) and treated with the sonicated TGR5 agonist (30 μ M INT-777), and cells cultured in normal glucose medium (5.5mM basal control) without any treatment added. When compared to the control (basal levels), GLP-1 mRNA quantification levels were observed to be higher in STC-1 cells treated with INT-777. Values are represented by the means \pm SEM in 3 independent experiments (n=3).

* $p < 0.05$, ** $p < 0.01$ vs basal.

Figure 10:



4. DISCUSSION

As Diabetes Mellitus and obesity continue to be serious health concerns globally, researchers will continue to study the mechanisms causing them. More importantly, research will look to develop innovative drugs and treatment therapies that counteract, or even cure, the associated complications. GLP-1 has continued to be a novel target for such research, as it truly has functions in improving glucose homeostasis. Along with GLP-1, the effecting bile acid receptors (TGR5 and FXR) have been studied to determine their effects on GLP-1 regulation. Numerous physiological functions of each of these receptors have already been discovered, with more discoveries made each year.

In summary, this study has demonstrated the high expression of GLP-1, TGR5, and FXR mRNA in the intestinal mucosa of mice. Not only are these receptors highly expressed in the small intestine, but they are also highly expressed in the colon as well. GLP-1, TGR5, and FXR were expressed in both wildtype and diabetic (db/db) mouse tissues. The receptors GLP-1 and TGR5 showed an increase in expression in db/db mouse intestine mucosa (small intestine and colon), when compared to the wildtype control. However, FXR showed a decrease in expression in diabetic mouse tissues, when compared to the wildtype.

Furthermore, this research has confirmed GLP-1, TGR5, and FXR expression in the enteroendocrine, Secretin Tumor Cell Line (STC-1). Even though STC-1 cells represent around 1% of cells in the intestinal tract, they still represent a reliable and easily reproducible enteroendocrine cell model. As such, the STC-1 cell models can be used to predict the roles of many gastrointestinal hormones in humans, as these cells share many of the same physiological functions and structures as native enteroendocrine cells in the intestines.²³

In addition to the detection of these receptors in STC-1 cells, GLP-1, TGR5, and FXR expression was found to be increased in cells cultured in hyperglycemic growing medium. GLP-1 quantification levels even revealed a linear increase with an increasing glucose concentration (5.5mM control to 25mM to 40mM) in growing medium of STC-1 cells, suggesting that the degree of hyperglycemia may have an effect on TGR5 and FXR as well. Hyperglycemia was tested in STC-1 cells to see if the receptor expression level change, noted in diabetic tissues, was specifically due to the rise of glucose concentrations.

GLP-1 and TGR5 showed a similar trend in both diabetic mouse tissues and in STC-1 cells, in that quantifications levels were significantly increased. This increase could be attributed to the effect that a rise in glucose concentration had on expression levels. It is important to note that mouse blood glucose levels were measured prior to experimentation, showing a 3 fold increase in diabetic mice when compared to wildtype mice. This plasma glucose fold change increase (3 fold) in mouse tissue is consistent with the fold change increase in STC-1 cell receptor expression. The predicted increase in hyperglycemic dependent expression occurred.

FXR, however, showed an opposite trend in expression in diabetic tissues and in the STC-1 cell line. The decreased expression in diabetic tissue and the increased expression in STC-1 cells could be attributed to many variances between the two. For example, db/db mice were exposed to chronic hyperglycemia while STC-1 cells only experienced acute hyperglycemia. Also, FXR expression differences could be due to a local response effect. FXR may increase in certain cell populations but decrease overall in whole intestine and whole colon samples. Furthermore, discrepancies have been reported between similar studies with the use of STC-1 cell models, as experimental conditions can be highly variable.²³

Lastly, STC-1 cells (containing 5.5mM glucose) treated for 48 hours with the TGR5 receptor agonist (INT-777) resulted in a significant increase in GLP-1 expression, demonstrating the connection between the two. This increase in GLP-1 expression further solidified the link between glucose concentrations, TGR5 activation and GLP-1 release in diabetes. An increase in glucose concentration led to an increase in TGR5 expression, which further led to an increase in GLP-1 expression (\uparrow [glucose] \rightarrow \uparrow TGR5 \rightarrow \uparrow GLP-1). In other words, TGR5 activity positively regulates and promotes GLP-1 expression. Once expressed or released, GLP-1 can provide its physiological benefits in aiding in the regulation of glucose homeostasis. Insulin will be released, while inhibition of glucagon will occur, thus leading to increased insulin sensitivity and altered diabetic outcomes.³⁰

Future research is still needed to fully understand the mechanisms and functions involved with these receptors, GLP-1, TGR5, and FXR. With the development of a more complete physiological image of these receptors, a more holistic healthcare strategy can be used to provide better patient outcomes and increase symptom relief. Specifically, future studies should include more research on the complexity of GLP-1 in relation to glucose metabolism, and more research on TGR5 and FXR localization and function.

REFERENCES

1. American Diabetes, A. (2015). (2) Classification and diagnosis of diabetes. *Diabetes Care*, 38 Suppl(Supplement_1), S8–S16. <https://doi.org/10.2337/dc15-S005>
2. Arora, T., Wegmann, U., Bobhate, A., Lee, Y. S., Greiner, T. U., Drucker, D. J., ... B??ckhed, F. (2016). Microbially produced glucagon-like peptide 1 improves glucose tolerance in mice. *Molecular Metabolism*, 5(8), 725–730. <https://doi.org/10.1016/j.molmet.2016.06.006>
3. Badalà, F., Nouri-mahdavi, K., & Raoof, D. A. (2008). NIH Public Access. *Computer*, 144(5), 724–732. <https://doi.org/10.1038/jid.2014.371>
4. Baghdasaryan, A., Claudel, T., Gumhold, J., Silbert, D., Adorini, L., Roda, A., ... Trauner, M. (2011). Dual farnesoid X receptor/TGR5 agonist INT-767 reduces liver injury in the Mdr2 -/- (Abcb4 -/-) mouse cholangiopathy model by promoting biliary HCO₃- output. *Hepatology*, 54(4), 1303–1312. <https://doi.org/10.1002/hep.24537>
5. Brighton, C. A., Rievaj, J., Kuhre, R. E., Glass, L. L., Schoonjans, K., Holst, J. J., ... Reimann, F. (2015). Bile acids trigger GLP-1 release predominantly by accessing basolaterally located G protein-coupled bile acid receptors. *Endocrinology*, 156(11), 3961–3970. <https://doi.org/10.1210/en.2015-1321>
6. Brunton, S. A. (2015). The potential role of sodium glucose co-transporter 2 inhibitors in the early treatment of type 2 diabetes mellitus. *International Journal of Clinical Practice*, 69(10), 1071–1087. <https://doi.org/10.1111/ijcp.12675>
7. Chen, X., Lou, G., Meng, Z., & Huang, W. (2011). TGR5: A novel target for weight maintenance and glucose metabolism. *Experimental Diabetes Research*, 2011. <https://doi.org/10.1155/2011/853501>
8. Chiang, J. Y. L. (2013). Bile acid metabolism and signaling. *Comprehensive Physiology*, 3(3), 1191–1212. <https://doi.org/10.1002/cphy.c120023>
9. Cipriani, S., Mencarelli, A., Palladino, G., & Fiorucci, S. (2010). FXR activation reverses insulin resistance and lipid abnormalities and protects against liver steatosis in Zucker (fa/fa) obese rats. *Journal of Lipid Research*, 51(4), 771–84. <https://doi.org/10.1194/jlr.M001602>
10. Collection, S. (2016). HHS Public Access, Immunogenetics of Type 1 Diabetes Mellitus 8(5), 583–592. <https://doi.org/10.1002/aur.1474.Replication>
11. Dai, F. F., Bhattacharjee, A., Liu, Y., Batchuluun, B., Zhang, M., Wang, X. S., ... Wheeler, M. B. (2015). A novel GLP1 receptor interacting protein ATP6ap2 regulates insulin secretion in pancreatic beta cells. *Journal of Biological Chemistry*, 290(41), 25045–25061. <https://doi.org/10.1074/jbc.M115.648592>

12. Dawson, P. A., & Karpen, S. J. (2015). Intestinal transport and metabolism of bile acids. *Journal of Lipid Research*, 56(6), 1085–1099. <https://doi.org/10.1194/jlr.R054114>
13. Gorgojo-Martínez, J. J., Serrano-Moreno, C., Sanz-Velasco, A., Feo-Ortega, G., & Almodóvar-Ruiz, F. (2017). Real-world effectiveness and safety of dapagliflozin therapy added to a GLP1 receptor agonist in patients with type 2 diabetes. *Nutrition, Metabolism and Cardiovascular Diseases*, 27(2), 129–137. <https://doi.org/10.1016/j.numecd.2016.11.007>
14. Guo, C., Chen, W.-D., & Wang, Y.-D. (2016). TGR5, Not Only a Metabolic Regulator. *Frontiers in Physiology*, 7(December), 646. <https://doi.org/10.3389/fphys.2016.00646>
15. Guo, C., Qi, H., Yu, Y., Zhang, Q., Su, J., Yu, D., ... Wang, Y. D. (2015). The G-protein-coupled bile acid receptor Gpbar1 (TGR5) inhibits gastric inflammation through antagonizing NF- κ B signaling pathway. *Frontiers in Pharmacology*, 6(DEC), 1–9. <https://doi.org/10.3389/fphar.2015.00287>
16. He, H. (2016). *Arteriosclerosis, Thrombosis, and Vascular Biology*, 36(11).
17. Houten, S. M., Watanabe, M., & Auwerx, J. (2006). Endocrine functions of bile acids. *The EMBO Journal*, 25(7), 1419–25. <https://doi.org/10.1038/sj.emboj.7601049>
18. Kumar, D. P., Asgharpour, A., Mirshahi, F., Park, S. H., Liu, S., Imai, Y., ... Sanyal, A. J. (2016). Activation of transmembrane bile acid receptor tgr5 modulates pancreatic islet - Cells to promote glucose homeostasis. *Journal of Biological Chemistry*, 291(13), 6626–6640. <https://doi.org/10.1074/jbc.M115.699504>
19. Lackey, D., Yoshihara, E., Perino, A., Jacinto, S., Lukashava, Y., Atkins, A. R., ... Evans, R. M. (2016). Reduces Obesity and Insulin Resistance, 21(2), 159–165. <https://doi.org/10.1038/nm.3760>. Intestinal FXR agonism promotes adipose tissue browning and reduces obesity and insulin resistance
20. Li, T., & Chiang, J. Y. L. (2014). Bile Acid Signaling in Metabolic Disease and Drug Therapy. *Pharmacological Reviews*, 66(4), 948–983. <https://doi.org/10.1124/pr.113.008201>
21. Maldonado-Valderrama, J., Wilde, P., MacIerzanka, A., & MacKie, A. (2011). The role of bile salts in digestion. *Advances in Colloid and Interface Science*, 165(1), 36–46. <https://doi.org/10.1016/j.cis.2010.12.002>
22. Manuscript, A., Signaling, B. A., & Regeneration, L. (2016). NIH Public Access, 1849(2), 196–200. <https://doi.org/10.1016/j.bbagr.2014.05.021>. Bile
23. Mccarthy, T., Green, B. D., Calderwood, D., Gillespie, A., Cryan, J. F., Giblin, L., ... Giblin, L. (2015). STC-1 Cells, 211, 211–220. https://doi.org/10.1007/978-3-319-16104-4_19

24. Meissner, M., Herrema, H., van Dijk, T. H., Gerding, A., Havinga, R., Boer, T., ... Kuipers, F. (2011). Bile acid sequestration reduces plasma glucose levels in db/db mice by increasing its metabolic clearance rate. *PLoS ONE*, 6(11), 1–8. <https://doi.org/10.1371/journal.pone.0024564>
25. Parker, H. E., Wallis, K., Le Roux, C. W., Wong, K. Y., Reimann, F., & Gribble, F. M. (2012). Molecular mechanisms underlying bile acid-stimulated glucagon-like peptide-1 secretion. *British Journal of Pharmacology*, 165(2), 414–423. <https://doi.org/10.1111/j.1476-5381.2011.01561.x>
26. Pols, T. W. H., Noriega, L. G., Nomura, M., Auwerx, J., & Schoonjans, K. (2011). The bile acid membrane receptor TGR5 as an emerging target in metabolism and inflammation. *Journal of Hepatology*, 54(6), 1263–1272. <https://doi.org/10.1016/j.jhep.2010.12.004>
27. Poole, D. P., Godfrey, C., Cattaruzza, F., Cottrell, G. S., Kirkland, J. G., Pelayo, J. C., ... Corvera, C. U. (2010). Expression and function of the bile acid receptor GpBAR1 (TGR5) in the murine enteric nervous system. *Neurogastroenterology and Motility*, 22(7), 1–21. <https://doi.org/10.1111/j.1365-2982.2010.01487.x>
28. Prawitt, J., Abdelkarim, M., Stroeve, J. H. M., Popescu, I., Duez, H., Velagapudi, V. R., ... Staels, B. (2011). Farnesoid X receptor deficiency improves glucose homeostasis in mouse models of obesity. *Diabetes*, 60(7), 1861–1871. <https://doi.org/10.2337/db11-0030>
29. Rizzo, G., Passeri, D., De Franco, F., Ciaccioli, G., Donadio, L., Rizzo, G., ... Adorini, L. (2010). Functional characterization of the semisynthetic bile acid derivative INT-767, a dual farnesoid X receptor and TGR5 agonist. *Molecular Pharmacology*, 78(4), 617–30. <https://doi.org/10.1124/mol.110.064501>
30. Sonne, D. P., Hansen, M., & Knop, F. K. (2014). Bile acid sequestrants in type 2 diabetes: potential effects on GLP1 secretion. *European Journal of Endocrinology / European Federation of Endocrine Societies*, 171(2). <https://doi.org/10.1530/EJE-14-0154>
31. Thomas, C., Gioiello, A., Noriega, L., Strehle, A., Oury, J., Rizzo, G., ... Schoonjans, K. (2010). NIH Public Access, 10(3), 167–177. <https://doi.org/10.1016/j.cmet.2009.08.001>. TGR5-mediated bile acid sensing controls glucose homeostasis
32. Trabelsi, M.-S., Daoudi, M., Prawitt, J., Ducastel, S., Touche, V., Sayin, S. I., ... Lestavel, S. (2015). Farnesoid X receptor inhibits glucagon-like peptide-1 production by enteroendocrine L cells. *Nature Communications*, 6, 7629. <https://doi.org/10.1038/ncomms8629>
33. Watanabe, M., Horai, Y., Houten, S. M., Morimoto, K., Sugizaki, T., Arita, E., ... Auwerx, J. (2011). Lowering bile acid pool size with a synthetic farnesoid X receptor (FXR) agonist induces obesity and diabetes through reduced energy expenditure. *Journal of Biological Chemistry*, 286(30), 26913–26920. <https://doi.org/10.1074/jbc.M111.248203>

- ³⁴. Zhang, Y., Lee, F. Y., Barrera, G., Lee, H., Vales, C., Gonzalez, F. J., ... Edwards, P. a. (2006). Activation of the nuclear receptor FXR improves hyperglycemia and hyperlipidemia in diabetic mice. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(4), 1006–1011. <https://doi.org/10.1073/pnas.0506982103>

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