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Regulation of Intestinal GLP-1 and GLUT2 genes underlie hypoglycemia in Desplatsia subericarpa (Bocq)-Fed Wistar Rats

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ABSTRACT

Background & Aim: Indigenous people of West Africa use the whole-leaf of *Desplatsia subericarpa* (Bocq) in anti-diabetic soup delicacy. This study was designed to validate the anti-diabetic claims and delineating possible mechanisms.

Experimental: RT-PCR method was used to investigate regulation of intestinal glucose transporter 2 (GLUT2) and glucagon-like peptide-1 (GLP-1), and pancreatic insulin, L-type voltage-gated calcium channel genes. Insulin exocytosis was also monitored using ELISA method. The kidney sample was investigated for biomarkers of injury (kidney injury molecule-1 (KIM-1) and interleukin-1-β (IL-1β)).

Results: GLP-1 up-regulation, GLUT2 down-regulation and increased insulin exocytosis but not increased insulin gene expression was observed in animals after a 3-day culinary exposure to *D. Subericarpa* leaves. This mechanism may explain hypoglycemia in streptozotocin-induced diabetes in animals in this study. KIM-1 and IL-1-β genes were marked up regulated in normal animals exposed (14-day) to *D. Subericarpa*.

Recommended applications/industries: *D. Subericarpa* whole leaf contains phytochemicals principles with anti-diabetic potency but may be nephrotoxic. Therefore, for clinical use, selective fractionation of active components from the toxic components is desirable.

1. Introduction

Desplatsia subericarpa is an understory dense tree growing up to 10 meters tall. The bole is about 4-10 cm in diameter. The glabrous papery leavesare alternate and simple with stipules.Drupe is 6-10cm long and 5-7.5 cm wide (Burkill, 2000; Keay, 1958). *D.Subericarpa* has been identified following an ethnopharmacological survey of neglected plants with food and therapeutic uses in Southern Nigeria. Folklorically, the fresh leaves of *D. subericarpa* are eaten as vegetables while the dried leavesaremade into a decoction useful in managing diabetic conditions yet

without scientific data to validate this claim and explain its mode of action, safety in humans.

Glucagon-like peptide 1 (GLP-1) is a potent incretinhormone produced in the L-cells of thedistal ileum and colon. In the L-cells, tissue-specific post-translational processing of the proglucagon gene generates GLP-1(Ezcurr *et al.,* 2013; Velasquez *et al.,* 2010), which acts onβ cells GLP-1 receptor, and mediates its glucose responsiveness by promoting insulin exocytosis in GLUT2 (the normally expressed high K_m glucose transporter of β cells)-dependent manner (Chen *et al.,* 1990). Indeed, GLP-1/GLP-1R signaling and GLUT2, mediate glucose-stimulated insulin secretion, and their blockage impair glucose sensing with resulting hyperglycemia (MacDonald *et al.*, 2002).

TGR5 is a bile acid activated G-protein coupled receptor, which plays key regulatory roles in energy expenditure and diet-induced obesity through intestinal (GLP-1) mechanisms (McKillop *et al.,* 2016; Pols *et al.,* 2010; Wacker *et al.,* 2014). Pharmacological targeting of TGR5 has been identified as a promising incretin-based strategy for managing type 2 diabetes and metabolic disorders such as non-alcoholic fatty liver disease. Therefore, in this study,TGR5/glucagon-like peptide-1 (GLP-1)/insulin pathway[11] along the intestino-hepato-pancreatic axis was investigated in *Desplatsia subericarpa* Bocq.-fed Wistar Rats. Furthermore, we investigated blood glucose regulation in streptozotocin-induced diabetic rats fed *Dsubericarpa* Bocq-based diet, and finally evaluate nephrotoxicity following sub-chronic exposure. Our results support intestinal GLP-1 up-Regulation and GLUT2 down-regulation in *Desplatsia subericarpa* Bocq.-fed Wistar Rats which may play significant role in its antidiabetic claims.

2. Materials and Methods

2.1   Plant materials

Desplatsia subericarpa Bocq. leaves were collected in January 2016 from Ugbogiobo village located in Ovia North Local Government Area of Edo State, Nigeria. Leaves were dried in a cool dry place for two weeks and oven died for 30 minutes at 40° C to ensure leaves were crispy. Dried leaves were pulverized using a mechanical mill, sieved and used as feed formulation as described below. A voucher specimen was deposited in the University of Benin Herbarium (UBHm0284).

2.2   Animal studies

6-week-old male inbred Wistar Rats were used with strict compliance with the NIH Guide for the Care and Use of Laboratory Animals. All the protocols were also reviewed and approved by Laboratory Animal Ethical Committee of AdekunleAjasin University, Akungba-Akoko. The Animals (now referred to as subjects) were maintained on a standard rat chow and water *ad libitum* for 5 days prior to grouping and experimentation.

2.3   D.   subericarpa-regulated   anti-diabetic transcriptional program along intestinal-pancreatic-hepatic axis

Subjects were housed three (3) per cage under a 12-h light/dark condition. Subject randomization and grouping for this study has been presented (fig.1) for clarity. 6-hr prior to sacrifice, food and water were withheld; anesthesia was induced in the subjects using chloroform. The Intestine, pancreas, liver and blood samples (cardiac puncture) were collected for further analysis.

2.4   Diabetes study

Diabetes was induced by intraperitoneal injection of single dose of freshly prepared solution of streptozotocin (in 0.1 M sodium citrate buffer, pH 4.5), 45 mg/kg) to overnight fasted subjects. Three days after administration, hyperglycemic subjects were grouped into diabetic control (100% feed/honey) and diabetic treatment groups (10% *D. subericarpa* leaf powder/90% rat chow mixed in honey), basal group was also fed like diabetic control but without streptozotocin treatment. Blood samples were taken three (3) times over of 9 feeding days for non-fasting blood glucose determination using the glucose oxidase method (Barham & Trinder, 1972).

2.5   Blood insulin quantification

Blood samples taken via cardiac puncture were stored in heparinized tubes, centrifuged at 3500 rpm for 15 minutes to separate the plasma for insulin

quantification using insulin ELISA kits (EZRMI-13K) according to the manufacturer's instructions.

2.6 *Total RNA isolation, Reverse transcription and PCR amplification*

RNA samples were extracted from tissues (n=3) using acid guanidiniumthiocyanate-phenol-chloroform extraction method. Genomic DNA contaminant was digested using DNase I (Fermentas, Ontario, Canada) treatment. The reaction mixture contains 2 µL of DNase I, 5 µL of reaction buffer and 23 µL RNA sample. The mixture was incubated at 37°C for 30 minutes. DNase I inactivation was achieved by adding 5 µL EDTA (25 mM) followed by 65°C incubation for 10 minutes[13]. RNA yield and purity were then spectrophotometrically determined. Prior to cDNA conversion, all RNA samples were diluted to 100 ng concentration using nuclease free water. MMLV Reverse Transcriptase 1st-Strand cDNA Synthesis Kit (NEB) was used for RNA-to-cDNA conversion according to the manufacturer's instruction. PCR amplification for the determination of genes whose primers (SnapGene software) are listed below was done using the following protocol: PCR amplification was performed in a total of 25 µl volume reaction mixture containing 2 µlcDNA (40 ng), 2 µl primer (100 pmol) 12.5 µl ReadyMix Taq PCR master mix (Sigma Aldrich) and 8.5 µl nuclease-free water. Initial denaturation at 95 °C for 5 minutes was followed by 20 cycles of amplification (denaturation at 95 °C for 30 seconds, annealing at 55 °C for 30 seconds and extension at 72 °C for 60 seconds) and ending with final extension at 72 °C for 10 minutes.

2.5 *Primers*

GLUT2F-5'-GACCTGGATTGAGACAACAACTCCG-3'
GLUT2R-5'-TATTATTACCTCTTGAGGTGCATTGATCACACC-3'
GLP-1-F-5'-TCCCAAAGGAGCTCCACCTG-3'
GLP-1-R-5'-TTCTCCTCCGTGTCTTGAGGG-3'
FAS-F-5'-AGAGACGGCAGCAGCG-3'
FAS-R-5'-GATCCTTCAGCTTTCCAGACCG-3'
G6PDD-F-5'-AAAGCACACGCCCTCTTGC-3'
G6PDD-R-5'-CTTGGTGGAAGGCATCACCC-3'
INSULIN-F-5'-AACCCTAAGTGACCAGCTACAATCA-3'
INSULIN-R-5'-AAACCACGTTCCCCACACAC-3'
LT-VDCC-F-5'-CTAGGCAAGCTCTCTACCAC-3'
LT-VDCC-R-5'-AGTAACCAGGTTGGTTCATG-3'
IL1☐-F-5'-GGCTCATCTGGGATCCTCTCC-3'
IL1☐-R-5'-GACACTGCCTTCCTGAAGCTC-3'
KIM-1-F-5'-GGTGCCTGTGAGTAAATAGATCA-3'
KIM-1-R-5'-TAAACTTCAACTACCTTAAACACAATAAGATG-3'
☐-Actin-F-5'-GTCGAGTCCGCGTCCAC-3'
☐-Actin-R-5'-AAACATGATCTGGGTCATCTTTTCACG-3'

2.6. *Image processing and statistical analysis*

In-gel amplicon bands (n=3) were captured using iPhone-5s camera (Noir), image post-processing was done on Keynote platform and band quantification was done using Image-J software. Data presented here expressed as Mean ± Standard Error of Mean (SEM). Statistical analyses were performed using t-test (P < 0.05 vs. basal control) using Graph Pad Prism version. 7 (GraphPad Software, Inc. La Jolla, CA, USA) software.

3. Results and discussion

3.1 *Intestinal GLP1 up-regulation and GLUT2 down-regulation following culinary administration of D. subericarpa*

Our understanding of ethnopharmacological uses of D. *subericarpa* leaves is infantile at this stage and to our knowledge; this study provides the first systematic study at unraveling its antidiabetic activity. First, our choice of administering the whole-leaf in culinary exposure was informed by the consensus that the leaf is traditionally made into vegetable decoction for managing diabetic complications. To mimic this, normal rat chow and the dried leaf powder were mixed

together in 90:10 and 70:30 ratios respectively. To aid consumption in animals, 100mL of pure honey was used to formulate the diet. The feed (Fig. 1) was administered *ad libitum* for 48 hr period. RT-PCR gel electrophoresis of RNA samples isolated from the intestinal crypt of the animals following feeding period showed up-regulated GLP-1 expression and down-regulated GLUT2 genes. GLUT2 is the major route for glucose absorption across the brush-border membrane (Kellett & Helliwell, 2000) and its inhibition has been identified as a antidiabetic mechanism in some plants extracts(Nistor Baldea *et al.,* 2010). Uncharacterized components of D. *subericarpa* leaves interfere with the expression of GLUT2, which appears more robust hypoglycemic mechanism than inhibition or blockage of mobilization to the brush-border.

We propose a plausible mechanism underlying GLUT2 gene downregulation as interference with sterol response element-binding protein (SREBP)-1c, which is known to facilitate glucose-stimulated GLUT2 gene expression (Im *et al.,* 2005). Note that direct inhibition of (SREBP)-1c has been chosen rather than inhibition membrane-bound transcription factor site-1 protease (S1P)(Brown & Goldstein, 1999), which catalyzes its post-translational maturation because direct inhibition of S1P would have resulted in hepatic downregulation of fatty acid synthase and glucose-6-phosphate dehydrogenase whose regulation are controlled by (SREBP)-1a(Inoue *et al.*, 2005). Our result show increased expression of hepatic fatty acid synthase and glucose-6-phosphate dehydrogenase (Fig. 3), providing clear evidence against S1P inhibition.

Next, GLP-1 expression pattern was investigated. GLP-1 is an enteroendocrine L-cells hormone produced following accumulating nutrients such as glucose within the gastrointestinal tract. Indeed, repression of GLUT2 should cause glucose accumulation within the gastrointestinal space resulting in increased GLP-1 expression and release, which in turn stimulates insulin and inhibits glucagon release with concomitant hypoglycemic effects (Ezcurra *et al.,* 2013; Velasquez

et al., 2010). Additionally, GLP-1 upregulation may proceed though TGR5 activation as naturally occurring TGR5 agonists have been previously shown to modulate GLP-1 biosynthesis and secretion(Jafri et al., 2016).*D. subericarpa* may contain TGR5 phytochemical agonists; some of these scaffolds have been characterized in plants such as *Olea europraea* (Sato *et al.,* 2007).

3.2 *Pancreatic Insulin release not gene expression is regulated by culinary administration of D. Subericarpa*

The desired goal in treatment of type-2 diabetes is to improve cell health (Marchetti *et al.*, 2009), increase insulin release, and tissue absorption of blood glucose via GLUT4, which is rapidly translocated to the membrane of target tissue downstream insulin/Insulin-receptor signaling pathway (Leto & Saltiel, 2012). We tested whether D. *subericarpa* leaves also alter insulin and L-type voltage gated channel transcription in the pancreas (Fig. 2D). While our data showed that none of these genes is significantly upregulated in the test groups (*vs.* control ($p < 0.05$), Fig. *2D, ii&iii*), blood insulin release is however dose-dependently increased in the treatment groups (Fig. 2D, *iv*). Increased blood insulin levels following *D. subericarpa* may have resulted from increased GLP-1 expression as stimulation of insulin secretion from pancreatic β-cells via glucagon-like peptide 1 receptor (GLP-1R)/GLP-1 (or other agonists) is well documented (Meloni *et al.,* 2013) Paradoxically, blunted GLUT2 expression may result in low blood glucose, which should act synergistically with GLP-1 to activate insulin release pathway in pancreatic β-cells (MacDonald *et al.,* 2002), clearly insulin release mediated by *D. subericarpa* may involve GLP-1 but glucose-independent manner. Interestingly, TGR5 agonists are the scaffolds, which mediate secretion of GLP-1 in the absence of glucose(Lan *et al.,* 2012), whether GLP-1 is sufficient for insulin release in the absence of glucose is yet to be determined.
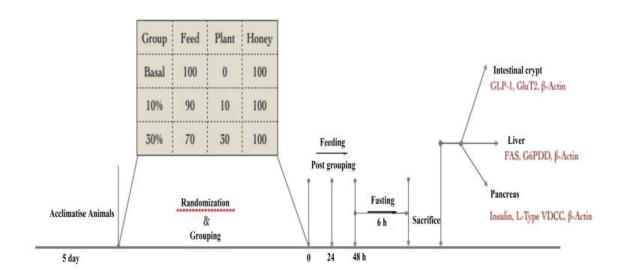
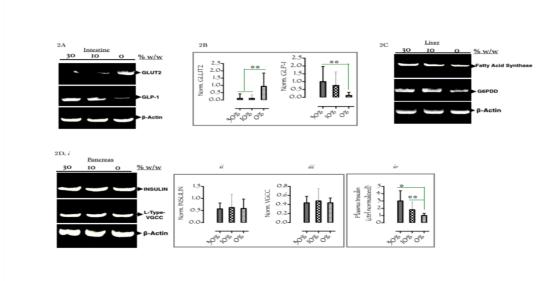**Fig. 1.** Experimental Design (normal rats)

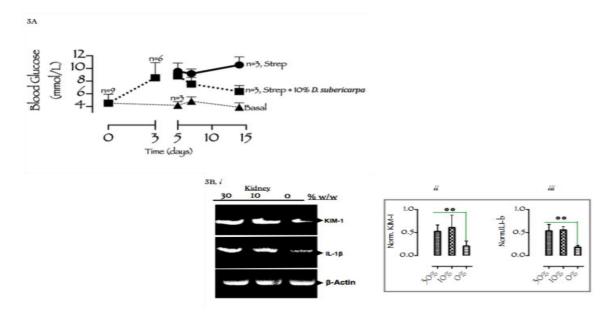**Fig. 2**. Querying anti-diabetic mechanism(s) of *D. subericarpa* leaves using gene expression studies.

(A)   Representative gel image showing intestinal mRNA levels of GLUT2, GLP-1and ꞵ actin.

(B)   Column depiction of ꞵ actin-normalized GLUT2 and GLP-1 levels in the intestine from band density estimate of n=3 independent samples.

(C)   Representative gel image showing liver mRNA levels of fatty acids synthase (FAS), glucose-6 phosphate dehydrogenase (G6PDD) and ꞵ actin.

(D)   (*i*) Representative gel image of pancreatic expression of insulin, L-type voltage-gated calcium channel (LT-VGCC) and ꞵ actin. Column representation of band density following quantification and normalization of insulin (*ii*) and LT-VGCC (*iii*). Column represents plasma insulin profile of experimental animals and control.

(All experiments were sampled in triplicate (n=3), ** (*p< 0.05vs. control*), * (*p< 0.01 vs. control*)

Fig. 3. *D. subericarpa* leaves exhibit anti-diabetic potency and toxicity in experimental animals (A). Line graph showing blood glucose profile with time in streptozotocin-induced hyperglycemia (treated and untreated) and basal control groups. (B, *i*) Gel image representation of mRNA levels of kidney toxicity biomarkers (Kidney injury molecule-1 (KIM-1) and interleukin-1 (IL-1)-□, column representation of band density following quantification and normalization of KIM-1 (*ii*) and IL1-□□ (*iii*) following □□actin normalization. (All experiments were sampled in triplicate (n=3), ** ($p < 0.05$ *vs. control*))

3.3   D. Subericarpa reverses hyperglycemia in experimental animals but exhibit sub-chronic nephrotoxicity

To test the usability of *D. subericarpa* leaves in experimental diabetic condition, nine (9) animals were subjected to similar initial feeding conditions (normal rat chow with honey base), six (6) of the animals were induced with diabetes using streptozotocin (single dose, 45 mg/kg, *i.p.)*; day-3 post streptozotocin treatment, hyperglycemia was observed in all the treatment groups. Three (3) of the diabetic animals were chosen for treatment (10% *D. subericarpa*) for 9 days. Blood samples were drawn periodically (once every 3 days) from the tail of the animals for blood glucose determination. Indeed, *D. subericarpa* leaves in the diet of diabetic rats partially reversed hyperglycemia associated with streptozotocin treatment (Fig. 3A). This result clearly supports the folkloric use of *D. subericarpa* leaves in diabetic condition. Since diabetes is treated chronically, it should be interesting to know how the kidney functions in a 14-day treatment period.

Here, kidney injury molecule 1 (KIM-1)(Andreucci *et al.*, 2016) and interleukin 1 □ expression(Faubel *et al.*, 2007) patterns were investigated as biomarkers for kidney damage. Surprisingly, both genes were upregulated in both test subjects (Fig. 3B,*i-iii*). This finding may not be absolutely peculiar to *D. subericarpa* leaves as consumption of mixed Chinese herbs have resulted in renal tubular acidosis with hypokalaemic paralysis, rhabdomyolysis and subsequent acute renal failure (Lee *et al.,* 1999).

In conclusion, we have demonstrated that *D. subericarpa* leaves have potent anti-diabetic principles acting via GLUT2 gene downregulation and GLP-1 upregulation mechanisms. We have also shown its applicability in animal model of diabetes. Its application in humans may be limited in whole-leaf form as renal IL-1□ and kidney injury molecule 1 (KIM-1) expression were significantly increased in animals administered *D. subericarpa* leaves. Thus, reciprocal regulation of GLP-1 and GLUT2 genes by *D. subericarpa* leaves provides preliminary indication

that the leaves contain TGR5 agonists, since TGR5 activation is upstream GLP-1 gene upregulation. Taking a cue from previous studies, we hypothesize that the unidentified compounds may likely be of sterol class especially the triterpenes, which has demonstrated potent TGR5 agonism *in vitro* and *in vivo*(Schaap *et al.,* 2014); GLUT2 downregulation in SREBP-dependent manner also lends credence to our speculation as sterols likewise regulate the activity of SREBP(Nohturfft *et al.,* 1999).It may be instructive to suggest immediate isolation and characterization of SREBP-1c inhibiting/TGR5 activating principle, which may be different from the nephrotoxic components for anti-diabetes drug development.

4. Conclusion

This study was designed to validate the anti-diabetic claims and delineating possible mechanisms by following the gene expression pattern of intestinal glucose transporter 2 (GLUT2), glucagon-like peptide-1 (GLP-1), pancreatic insulin, L-type voltage-gated calcium channel genes. Similarly, The kidney sample was investigated for biomarkers of injury (kidney injury molecule-1 (KIM-1), interleukin-1-β (IL-1β)).

Upregulated GLP-1, increased insulin exocytosis but not increased insulin gene expression coupled with GLUT2 down-regulation following culinary exposure to *D. Subericarpa* leaves solidly support its anti-diabetic claims. The anti-diabetic pharmacology may be blunted by nephrotoxicity as KIM-1 and IL-1-β genes were marked up regulated in normal animals exposed (14-day) to *D. Subericarpa*. We therefore report that the whole leaf indeed *D. Subericarpa* contains phytochemical principles with potent anti-diabetic potency but may be nephrotoxic; for future clinical use, selective fractionation of active components from the toxic components is desirable.

5. Acknowledgements

6. References

Andreucci, M., Faga, T., Riccio, E., Sabbatini, M., Pisani, A., & Michael, A. 2016. The potential use of biomarkers in predicting contrast-induced acute kidney injury. *Int J Nephrol Renovasc Dis, 9*, 205-221.

Barham, D., & Trinder, P. 1972. An improved colour reagent for the determination of blood glucose by the oxidase system. *Analyst, 97*(151), 142-145.

Brown, M. S., & Goldstein, J. L. 1999. A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. *Proc Natl Acad Sci U S A, 96*(20), 11041-11048.

Burkill, H. M. 2000. *The useful plants of West Africa.* (2 ed. Vol. 5). United Kingdom: families S-Z, Addenda. Royal Botanic Gardens, Kew, Richmond,.

Chen, L., Alam, T., Johnson, J. H., Hughes, S., Newgard, C. B., & Unger, R. H. (1990. Regulation of beta-cell glucose transporter gene expression. *Proc Natl Acad Sci U S A, 87*(11), 4088-4092.

Ezcurra, M., Reimann, F., Gribble, F. M., & Emery, E. 2013. Molecular mechanisms of incretin hormone secretion. *Curr Opin Pharmacol, 13*(6), 922-927.

Faubel, S., Lewis, E. C., Reznikov, L., Ljubanovic, D., Hoke, T. S., Somerset, H., . . . Edelstein, C. L. 2007. Cisplatin-induced acute renal failure is associated with an increase in the cytokines interleukin (IL)-1beta, IL-18, IL-6, and neutrophil infiltration in the kidney. *J Pharmacol Exp Ther, 322*(1), 8-15.

Im, S. S., Kang, S. Y., Kim, S. Y., Kim, H. I., Kim, J. W., Kim, K. S., & Ahn, Y. H. 2005. Glucose-stimulated upregulation of GLUT2 gene is mediated by sterol response element-binding protein-1c in the hepatocytes. *Diabetes, 54*(6), 1684-1691.

Inoue, N., Shimano, H., Nakakuki, M., Matsuzaka, T., Nakagawa, Y., Yamamoto, T., . . . Yamada, N. 2005. Lipid synthetic transcription factor SREBP-1a activates p21WAF1/CIP1, a universal cyclin-

dependent kinase inhibitor. *Mol Cell Biol, 25*(20), 8938-8947.

Jafri, L., Saleem, S., Calderwood, D., Gillespie, A., Mirza, B., & Green, B. D. 2016. Naturally-occurring TGR5 agonists modulating glucagon-like peptide-1 biosynthesis and secretion. *Peptides, 78*, 51-58.

Keay, R. W. J. 1958. *Flora of West Africa* (2 ed. Vol. 1, part 2). London, United Kingdom: Crown Agents for OverseaGovernment and Administrations.

Kellett, G. L., & Helliwell, P. A. 2000. The diffusive component of intestinal glucose absorption is mediated by the glucose-induced recruitment of GLUT2 to the brush-border membrane. *Biochem J, 350 Pt 1*, 155-162.

Lan, H., Lin, H. V., Wang, C. F., Wright, M. J., Xu, S., Kang, L., . . . Kowalski, T. J. 2012. Agonists at GPR119 mediate secretion of GLP-1 from mouse enteroendocrine cells through glucose-independent pathways. *Br J Pharmacol, 165*(8), 2799-2807.

Lee, C. T., Wu, M. S., Lu, K., & Hsu, K. T. 1999. Renal tubular acidosis, hypokalemic paralysis, rhabdomyolysis, and acute renal failure--a rare presentation of Chinese herbal nephropathy. *Ren Fail, 21*(2), 227-230.

Leto, D., & Saltiel, A. R. 2012. Regulation of glucose transport by insulin: traffic control of GLUT4. *Nat Rev Mol Cell Biol, 13*(6), 383-396.

MacDonald, P. E., El-Kholy, W., Riedel, M. J., Salapatek, A. M., Light, P. E., & Wheeler, M. B. 2002. The multiple actions of GLP-1 on the process of glucose-stimulated insulin secretion. *Diabetes, 51 Suppl 3*, S434-442.

Marchetti, P., Lupi, R., Del Guerra, S., Bugliani, M., D'Aleo, V., Occhipinti, M., . . . Masini, M. 2009. Goals of treatment for type 2 diabetes: beta-cell preservation for glycemic control. *Diabetes Care, 32 Suppl 2*, S178-183.

McKillop, A. M., Moran, B. M., Abdel-Wahab, Y. H., Gormley, N. M., & Flatt, P. R. 2016. Metabolic effects of orally administered small-molecule agonists of GPR55 and GPR119 in multiple low-dose streptozotocin-induced diabetic and incretin-receptor-knockout mice. *Diabetologia.* 59(12):2674-85.

Meloni, A. R., DeYoung, M. B., Lowe, C., & Parkes, D. G. 2013. GLP-1 receptor activated insulin secretion from pancreatic beta-cells: mechanism and glucose dependence. *Diabetes Obes Metab, 15*(1), 15-27.

Nistor Baldea, L. A., Martineau, L. C., Benhaddou-Andaloussi, A., Arnason, J. T., Levy, E., & Haddad, P. S. 2010. Inhibition of intestinal glucose absorption by anti-diabetic medicinal plants derived from the James Bay Cree traditional pharmacopeia. *J Ethnopharmacol, 132*(2), 473-482.

Nohturfft, A., DeBose-Boyd, R. A., Scheek, S., Goldstein, J. L., & Brown, M. S. 1999. Sterols regulate cycling of SREBP cleavage-activating protein (SCAP) between endoplasmic reticulum and Golgi. *Proc Natl Acad Sci U S A, 96*(20), 11235-11240.

Pols, T. W., Auwerx, J., & Schoonjans, K. 2010. Targeting the TGR5-GLP-1 pathway to combat type 2 diabetes and non-alcoholic fatty liver disease. *Gastroenterol Clin Biol, 34*(4-5), 270-273.

Sato, H., Genet, C., Strehle, A., Thomas, C., Lobstein, A., Wagner, A., . . . Saladin, R. 2007. Anti-hyperglycemic activity of a TGR5 agonist isolated from Olea europaea. *Biochem Biophys Res Commun, 362*(4), 793-798.

Schaap, F. G., Trauner, M., & Jansen, P. L. 2014. Bile acid receptors as targets for drug development. *Nat Rev Gastroenterol Hepatol, 11*(1), 55-67.

Velasquez, D. A., Beiroa, D., Vazquez, M. J., Romero, A., Lopez, M., Dieguez, C., & Nogueiras, R. 2010. Central GLP-1 actions on energy metabolism. *Vitam Horm, 84*, 303-317.

Wacker, D. A., Wang, Y., Broekema, M., Rossi, K., O'Connor, S., Hong, Z., . . . Robl, J. A. 2014. Discovery of 5-chloro-4-((1-(5-chloropyrimidin-2-yl)piperidin-4-yl)oxy)-1-(2-fluoro-4-(methylsulfonyl)phenyl)pyridin-2(1H)-one (BMS-903452), an antidiabetic clinical candidate targeting GPR119. *J Med Chem, 57*(18), 7499-7508.