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Naturally-occurring TGR5 agonists modulating glucagon-like peptide-1 biosynthesis and secretion.

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Highlights

- Crude extracts of the plant *Fagonia cretica* stimulate GLP-1 and GIP secretion
- Ethyl acetate partitioning significantly enriches GLP-1 secretory activity
- Quinovic acid (QA) & 2 QA derivatives are potent & selective GLP-1 secretagogues
- QA and its derivatives stimulate GLP-1 secretion by activation of TGR5 receptors

Abstract

Selective GLP-1 secretagogues represent a novel potential therapy for type 2 diabetes mellitus. This study examined the GLP-1 secretory activity of the ethnomedicinal plant, *Fagonia cretica*, which is postulated to possess anti-diabetic activity. After extraction and fractionation extracts and purified compounds were tested for GLP-1 and GIP secretory activity in STC-1 pGIP/neo cells. Intracellular levels of incretin hormones and their gene expression were also determined. Crude *F. cretica* extracts stimulated both GLP-1 and GIP secretion, increased cellular hormone content, and upregulated gene expression of proglucagon, GIP and prohormone convertase. However, ethyl acetate partitioning significantly enriched GLP-1 secretory activity and this fraction underwent bioactivity-guided fractionation. Three isolated compounds were potent and selective GLP-1 secretagogues: quinovic acid (QA) and two QA derivatives, QA-3β-O-β-D-glycopyranoside and QA-3β-O-β-D-glucopyranosyl-(28→1)-β-D-glucopyranosyl ester. All QA compounds activated the TGR5 receptor and increased intracellular incretin levels and gene expression. QA derivatives were more potent GLP-1 secretagogues than QA. This is the first time that QA and its naturally-occurring derivatives have been shown to activate TGR5 and stimulate GLP-1 secretion. These data provide a plausible mechanism for the ethnomedicinal use of *F. cretica* and may assist in the ongoing development of selective GLP-1 agonists.

**Key words:** GLP-1, GIP, incretin, secretagogue, TGR5.
1. Introduction

Pharmacological interventions targeting the enteroinsular axis are a clinically-proven approach for improving glucose homeostasis in patients with type 2 diabetes mellitus [20]. Clinically approved pharmacological strategies include mimetics and analogues of glucagon-like peptide-1 (GLP-1), but also inhibitors of dipeptidylpeptidase-4 (DPP-4i or gliptins) which act to prevent the physiological breakdown of GLP-1 and its sister hormone glucose-dependent insulinotropic polypeptide (GIP) [7, 11-12, 20]. A third, more unestablished strategy is the enhancement of postprandial GLP-1 secretion by means of specific secretagogues [25]. The functioning of the enteroinsular axis is markedly affected by the onset of type 2 diabetes. On one hand there is clear evidence of impaired GIP action but not GIP secretion [15, 23, 34-35]. On the other hand defective or blunted GLP-1 secretion has been reported in type 2 diabetes on some occasions [23, 33-35]. Despite these observations of impaired GLP-1 secretion in type 2 diabetes GLP-1-based therapies retain an ability to stimulate insulin secretion from pancreatic beta-cells [15]. One future alternative to GLP-1 analogue/mimetic therapy could involve ways of selectively increasing endogenous GLP-1 secretion. This approach could potentially even be used in combination with gliptin therapies in order to further enhance levels of active endogenous GLP-1.

There is evidence that enhancement of endogenous postprandial GLP-1 secretion is an effective strategy. For example, dosing with the GLP-1 secretagogue L-Arginine increases circulating GLP-1 levels in both lean and obese mice, and it substantially improves glucose clearance [5]. GLP-1 receptor signalling appears to be pivotal since improvements in glucose tolerance are significantly less effective in GLP-1R<sup>−/−</sup> mice than wild-type littermates [5]. A number of other dietary-based GLP-1 secretagogues have been reported [4, 13, 17, 22, 26] but pharmacologically there is a focus on targeting several intestinal GPCRs believed to be important in modulating gut hormone secretion. These include the bile acid receptor TGR5.
[25] and fat-sensing receptors GPR40, GPR119, and GPR120 [38] all of which are present on the surface of enteroendocrine cells.

The present study investigated the incretin hormone secretory activity of the plant *Fagonia cretica* which was identified by routine systematic screening of plant materials. Although there is negligible scientific evidence that *F. cretica* possesses anti-diabetic activity [29] it is noteworthy that it is reportedly used in natural folk/Greco-Arab medicine for the treatment of diabetes [1, 3, 9]. Medicinal plants have been used to treat diabetes for millennia, and they offer a natural resource of anti-diabetic products for traditional ethnomedical systems in Asia and Africa. The prevalence of type 2 diabetes is rising fastest among developing countries [16] where 75-80% of the population relies on traditional herbal medicines for their primary healthcare [18]. Hence, there is a pressing need for the scientific characterisation of the numerous anti-diabetic medicinal plants described in traditional ethnomedical systems worldwide. There are a number of examples of commonly used clinical anti-diabetic therapies which are based on natural products. For example, the most commonly used anti-diabetic drug metformin is based on the discovery of galegine in the plant *Galega officinalis* [2]. Furthermore, the widely used anti-diabetic drug acarbose (an alpha glucosidase inhibitor) was discovered in a bacterium [32]. The present study sought to identify novel ethnobotanical compounds which preferentially stimulate GLP-1 secretion but not GIP secretion.

2. Materials and Methods

2.1 Plant material

An ongoing programme of work involving systematic screening of plant materials for incretin secretory activity led us to focus on *Fagonia cretica* (Synonym *F. indica*). The fresh aerial parts of plants were collected in Pakistan in September 2010. *F. cretica* (locally referred to as
Dhamasa) was collected from Mianwali. Plant species identification was carried out by Professor Dr. Rizwana Aleem Qureshi, Department of Plant Sciences, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan. Voucher specimens (HMP-461) were deposited in the “Herbarium of Medicinal Plants of Pakistan”, Quaid-i-Azam University, Islamabad, Pakistan.

2.2 Extraction and isolation of F. cretica compounds

The fresh aerial parts of F. cretica were rinsed with water, dried and crushed to dry weight to yield 22 kg of plant material. Crude plant extract (FCC) was prepared by maceration in a methanol-chloroform (1:1) solution for 7 days at room temperature. Extracts were filtered, concentrated with a rotary evaporator (45°C) under vacuum and the resulting crude was suspended in water. The water suspension was partitioned three times with n-hexane to obtain an n-hexane fraction (FCN). The residual aqueous suspension was then partitioned with ethyl acetate to obtain an ethyl acetate (FCE) fraction and an aqueous fraction (FCA). Each of the above fractions were concentrated in a rotary evaporator. A total of 3 compounds with GLP-1 secretory activity were isolated from FCE. All 3 compounds were obtained by chromatographic separation on a silica gel column, and their isolation and identification has previously been described in detail [29]. Compounds were identified by a combination of mass spectrometry and NMR spectroscopy (Bruker AVANCE 400 MHz NMR). Chemical structures were confirmed by comparison of their chemical and spectroscopic properties (as previously reported [29]). The compounds were: quinovic acid (QA), quinovic acid-3β-O-β-d-glycopyranoside (dQA), and quinovic acid-3β-O-β-d-glucopyranosyl-(28→1)-β-d-glucopyranosyl ester (EdQA). Structures of QA, dQA and EdQA can be found in the Supplementary Appendix (Figure 1).
2.3 Cells

pGIP/Neo STC-1 cells were a gift from Dr. B. Wice (Washington University of St. Louis) [28] with permission from Dr D. Hanahan (University of California, San Francisco, CA). These cells secrete both GLP-1 and GIP and are responsive to nutrient stimulation and are potentially a very useful model for studying the secretory responses of both incretin hormones [10, 14, 26]. DMEM culture medium containing 4.5 g/l with L-glutamine, without sodium pyruvate (Life Technologies, Paisley, UK) and supplemented with 10% foetal bovine serum, 100 U/ml penicillin, 100 mg/l streptomycin and geneticin - G418, 400 μg/ml purchased from Sigma (Dorset, UK). pGIP/Neo STC-1 cells were cultured in culture medium and incubated in a 5% CO₂ humidified atmosphere at 37°C. Cells underwent passage upon reaching 80–90% confluence and were used for studies between passage numbers 15–50.

2.4 GLP-1 and GIP Secretion

Cells were seeded into 12-well plates (2 x 10⁶ per well) and cultured overnight at 37 °C in a humidified atmosphere of 5% CO₂. Medium was removed and cells washed 3 times with HEPES buffer (20 mM HEPES, 10 mM glucose, 140 nM NaCl, 4.5 mM KCl, 1.2 mM CaCl₂, 1.2 mM MgCl₂) then allowed to incubate for 1h in HEPES buffer prior to adding fresh HEPES buffer (vehicle control) or HEPES buffer supplemented with test agents. Cells were incubated for 3 h after which the vehicle control and test agents were removed, centrifuged and stored at -80°C prior to ELISA assays. GLP-1 and GIP ELISA kits were purchased from Millipore (Billerica, MA, USA). GLP-1 assays detected only active forms of GLP-1 (7-36 amide and 7-37) whilst GIP assays detected total GIP (GIP (1-42) and GIP (3-42).

2.5 Cellular GLP-1 and GIP content

Cellular GLP-1 and GIP peptide levels were determined in pGIPneoSTC-1 cells after incubations with plant extract/compounds. Initially cells (2 x 10⁶) were seeded into 12 well
plates and cultured overnight at 37°C in a humidified atmosphere of 5% CO₂. Medium was removed and replaced with 1 ml of medium supplemented with 50 µM of the plant extract/compound. Following incubation the medium was removed and cells were washed with 1ml of HEPES buffer and GLP-1 and GIP were extracted by the addition of acid/ethanol (1.5% HCl (v/v): 75% ethanol (v/v): 23.5% H₂O (v/v)) and incubated overnight at 4°C. Acid/ethanol solutions were removed, centrifuged (2000 g, 5 min) to remove cellular debris, and the ethanol evaporated off using a SpeedVac sample concentrator (Genevac, Ipswich, UK). Samples were reconstituted in buffer and stored at -80°C prior to measurement by ELISA.

2.6 Real-time PCR

Cells (4 x 10⁶ per well) were seeded into 6-well culture plates with 1 ml of culture medium, allowed to attach overnight (24h; 37°C; 5%CO₂). Medium was removed, the cells were washed with HEPES buffer and then underwent pre-incubation with HEPES buffer (60 min). Buffer was aspirated off and cells were incubated for 3h with 400μl of plant extracts (250 µg/ml) and isolated compounds (50 µM). The vehicle control was unsupplemented HEPES buffer. After the test period samples were removed and the cells were trypsinised, centrifuged to obtain a pellet. RNA was isolated from pellet by using RNeasy Mini Kit (Qiagen). The purity of isolated RNA was determined using UV absorption at 260 and 280 nm. The cDNA was synthesized from 2µg of total RNA using dNTP, random primers and reverse transcriptase (High capacity cDNA reverse transcription kits, Applied Biosystems). Real-time PCR was performed using SYBR green PCR master mix and a SYBR green Real-time PCR reagents kit (Applied Biosystems). Sequences of custom synthesis primers (Invitrogen) used to amplify the cDNAs of interest can be found in the Supplementary Appendix (Table 1). All reactions were performed in Eco™ Real-Time PCR System with Eco™ software v3.1.7.0 (Illumina) and underwent PCR (95°C for 10 min; then 40 cycles of denaturation at 95°C for 30 sec, annealing at 61°C for 30 sec, and extension at 72 °C 30 sec).
2.7 Measurement of cytotoxicity and cell numbers

Cells were seeded into 96-well plates (5 x 10⁴ per well) and cultured overnight at 37 °C in a humidified atmosphere of 5% CO₂. Cells were exposed to test agents for 3 h after which medium and test agents were removed from the wells, Alamar blue (Invitrogen, NY, USA) added (100µl), the plate incubated (37°C; 1h) and absorbance read (570 - 600 nm) using a microplate reader (Tecan, Safire2). Parallel incubations were set up for measurement of cell numbers but after media was removed cells were fixed with 10% formalin for 10 min at room temperature. Cells were washed (x4) with PBS and permeabilization solution (0.25% Triton X-100 in PBS) added and incubated for 30 min at room temperature. Cells were washed with PBS (3 times x 5 min) in the dark after which Hoechst staining solution was added for 10 min. Cells were washed with PBS (x1) and PBS added to each well and the plate was sealed prior to fluorescent imaging 350-461 nm with an ArrayScan high content analysis system (Thermo, Loughborough, UK).

2.8 Measurement of TGR5 receptor activation

Stimulation of cAMP response was measured in CHO cells stably expressing human TGR5 (GPBAR1) via cAMP Hunter™ eXpress assay kit (DiscoveRx Corporation, Birmingham, UK). In brief, cells re-suspended (10,000 in 10 µl/well) in Hank’s balanced salt solution (137 mM NaCl, 5.4 mM KCl, 0.25 mM Na2HPO4, 0.44 mM KH2PO4, 1.3 mM CaCl2, and 1.0 mM MgSO4) with 25 mM HEPES and 0.01% (w/v) BSA were stimulated in the presence of plant extracts and compounds (5 µl/well) in a 96-well plate at room temperature for 30 min before adding antibody and lysis reagents according to manufacturer’s protocol. The plates were further incubated in the dark overnight after adding detection solution, and read using a Mithras LB940 Luminometer (Berthold Technologies, Germany) for 1 min per plate. Data were expressed as Relative Luminescence Unit (RLU) and forskolin (5 μM; 5 µl/well) was used as a positive control.
2.9 Statistical analysis

Experimental data were expressed as mean ± SEM and analyzed by one-way ANOVA with Tukey’s post-hoc test (GraphPad Prism version 5). P values less than 0.05 were deemed statistically significant.

3. Results

3.1 *F. cretica* plant extracts stimulate incretin hormone secretion and gene expression.

*F. cretica* extracts (125-250µg/ml) significantly stimulated GLP-1 secretion (3-55-fold; P<0.05; Figure 1A-B). GIP secretion was also significantly stimulated by *F. cretica* plant extracts although the effect was more modest (1.6-2.2-fold; P<0.05 Figure 1C-D). Gene expression of proglucagon (2.2-6.4-fold; P<0.001 Figure 2A), GIP (6.2-12.3-fold; P<0.001; Figure 2B) and PC1/3 (1.8-2.4-fold; P<0.001; Figure 2C) were all increased by exposure of cells to *F. cretica* extracts, and cell viability was largely unaffected (Figure 2D) except for FCA. Solvent partitioning of FCC with ethyl acetate (FCE) enriched GLP-1 secretory activity (41-55-fold) and proglucagon gene expression (6.4-fold) to the greatest extent. Contrastingly, no parallel improvement in GIP secretion was observed with FCE compared with FCC. For this reason FCE underwent further bioactivity-guided chromatographic separation.

3.2 Isolated *F. cretica* compounds stimulate GLP-1 secretion, upregulate incretin-related gene expression and increase cellular hormone content.

The 3 isolated compounds QA, dQA and EdQA were all capable of significantly stimulating GLP-1 secretion (Figure 3A-B), without triggering GIP secretion (Figure 3C-D). The most potent secretagogue appeared to be EdQA. EdQA was the only compound to significantly increase GLP-1 secretion at 25µM (Figure 3A; 1.6-fold; P<0.05), and at a concentration of 50 µM it gave a much GLP-1 secretory response (Figure 3B; 16.9-fold; P<0.001) than either QA
or dQA (5.8 and 8.7-fold, respectively; P<0.001). QA modestly upregulated the expression of the proglucagon gene (Figure 4A; 50µM; 2.4-fold; P<0.01) but did not affect the expression of GIP or prohormone convertase (PC1/3) genes (Figure 4B-C). Contrastingly, dQA and EdQA increased the expression of proglucagon (4.6 and 7.9-fold, respectively; p<0.001), GIP (1.9 and 2.2-fold, respectively; p<0.001) and PC1/3 genes (1.8 and 3.4-fold, respectively; p<0.001). In all cases EdQA increased gene expression by the greatest extent. None of the compounds had adverse effects on cell viability, but in the case of EdQA a slight improvement was noted (Figure 4D; 1.1-fold; P<0.05) along with a significant increase in both the GLP-1 and GIP cellular content (Figure 4F-G; 1.3 and 2.1-fold, respectively; P<0.01). Cell numbers were unaffected by any treatments (Figure 4E).

3.3 Isolated F. cretica compounds activate the TGR5 receptor

All 3 isolated compounds were capable of significantly increasing cAMP production in a TGR5 overexpressing cell-line (Figure 5). At a concentration of 100 µM QA, dQA and EdQA increased cAMP production by 1.6, 1.6 and 1.5-fold, respectively (P<0.001). EdQA was the only compound to significantly increase cAMP production at 33µM (1.2-fold; P<0.05).

4. Discussion

This investigation isolated and identified three novel GLP-1 secretagogue compounds occurring in a plant reportedly used in traditional ethnomedical systems to treat diabetes. Our focus on F. cretica was prompted by the results of screening studies which found that crude extracts potently stimulated incretin hormone secretion. A crude F. cretica extract potently stimulated the secretion of both GLP-1 and GIP, and increased the cellular levels of these hormones. The extract also increased the expression of proglucagon, GIP and PC1/3 genes. The aim of the study was to identify compounds which preferentially stimulate GLP-1
secretion. Therefore, bioactivity-guided fractionation was performed on the fraction yielded by ethyl acetate partitioning due to the observed enrichment in activity. In fact partitioning with ethyl acetate increased GLP-1 secretory activity more than 2-fold, and quite remarkably the overall GLP-1 concentrations after exposure were 41-55-fold higher compared with the vehicle control. In contrast, similar partitioning of the extract did not improve GIP secretion. The 3 compounds isolated and identified QA, dQA and EdQA all potently stimulated GLP-1 secretion, but importantly not GIP secretion. Their ability to activate the TGR5 receptor was subsequently confirmed by measurement of intracellular cAMP production in CHO cells transfected with TGR5 (Figure 5). As far as we are aware QA has not previously been reported to possess either GLP-1 secretory activity or TGR5 agonism. There are a variety of G protein-coupled receptors (GPCR) which play a role in modulating GLP-1 secretion, but TGR5 (GPBAR1) appeared to be the most logical target for these QA-derived compounds for several reasons. Firstly, the compounds appeared to stimulate cAMP in STC-1 pGIP neo cells (data not shown). GLP-1 secretion both in vitro and in vivo has been linked to Gs-coupled GPCRs which increase cAMP and activate PKA [30]. TGR5 is one such GPCR which was first discovered in 2002 and apparently acts physiologically as the primary mediator of bile-acid induced GLP-1 release [19, 21]. Pharmacologically-designed TGR5 agonist molecules are potent GLP-1 secretagogues and they appear to enhance L-cell responses to Ca\(^{2+}\) and glucose-induced GLP-1 secretion [24]. Secondly, STC-1 cells (of which STC-1 pGIP neo cells are a sub-clone) express significant amounts of TGR5 and the application of a TGR5 agonist to STC-1 cells increases the gene expression of PC1/3 [37] which was also upregulated in the present study. Thirdly, and perhaps most importantly QA is a triterpenoid compound and other naturally-occurring triterpenoid compounds have been shown to activate TGR5. For example, oleanolic acid is a triterpenoid extracted from *Olea europaea* leaves which stimulates GLP-1 secretion and possesses an antihyperglycaemic effect [27, 31]. Finally, it is worth mentioning that dQA and EdQA isolated here, are QA compounds derivatised at the C-3 and C-17 carbon
positions. Since dQA and EdQA (especially EdQA) possessed greater GLP-1 secretory activity than QA, derivatisation at these positions can be generally viewed as beneficial. Such an observation appears to be analogous to the structure-activity relationship studies of [8] who found that the TGR5 receptor activity of betulinic acid (another natural triterpenoid) could be improved by modification at the same carbon positions where dQA and EdQA are derivatised. In this regard QA could be viewed as an alternative chemical scaffold for the discovery and development of novel pharmacological TGR5 agonists – a class of compounds which is undergoing pre-clinical development as potential orally-deliverable therapies for type 2 diabetes [6, 25]. In addition to stimulating GLP-1 secretion TGR5 agonists offer other potential benefits for type 2 diabetic patients. Most notably they increase energy expenditure by increasing oxidative phosphorylation in the mitochondria of brown fat cells and skeletal muscle cells [22, 36]. It has been postulated that TGR5 therefore could have additional benefits in type 2 diabetes mellitus because patients have disproportionately higher levels of obesity [25].

Before optimal dosages for *F.cretica* can be determined quantitative analytical measurement of the QA compounds in *F.cretica* should to be conducted. However, based on the data obtained during the original fractionation of *F.cretica*, approximately 13mg of these QA compounds were isolated from 100g of an ethyl acetate extract (FCE) [29]. In vivo dose ranging studies should be conducted for *F.cretica* with measurement of plasma GLP-1 and plasma glucose as endpoints.

5. **Conclusion**

In conclusion, we have discovered three naturally occurring TGR5 agonists which potently stimulate GLP-1 secretion. The compounds provide a novel and plausible mechanism supporting the ethnomedicinal use of *F. cretica* in traditional ethnomedical systems.
Furthermore, the results indicate that QA could be used a potential chemical scaffold for the discovery and development of pharmacological TGR5 agonists, which are currently under investigation for the treatment of type 2 diabetes mellitus [25]. Structure-activity related studies of QA are clearly warranted. Finally, there is a need for in vivo studies in diabetic animal models to scientifically validate the potential glucose-lowering effects of F. cretica. This is especially interesting given that compounds in F. cretica including QA derivatives have modest inhibitory activity against DPP-4 [29] which may contribute to GLP-1 duration of action.

Contributor statements
Laila Jafri designed the experiments, analysed and interpreted the data and drafted the manuscript. Samreen Saleem, Danielle Calderwood and Anna Gillespie designed the experiments, analysed and interpreted the data. Brian Green and Bushra Mirza conceived the study, designed the experiments, analysed and interpreted the data, and drafted the manuscript. All authors approved the final version of the manuscript to be published.

Declaration of interests
None declared.

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References


Figure Legends

Figure 1. **Effects of *F. cretica* plant extracts on GLP-1 and GIP secretion.** STC-1 pGIPneo cells were incubated for 3h before determination of (A-B) GLP-1 secretion and (C-D) GIP secretion. Crude plant extract (FCC) was prepared by maceration in methanol-chloroform (1:1) and other extracts were prepared by partitioning with n-hexane (FCN), ethyl acetate (FCE) and aqueous (FCA) solvent phases. Results are mean ± SEM (n=3). *P<0.05, **P<0.01 and ***P<0.001.

Figure 2. **F. cretica** plant extracts influence incretin hormone biosynthesis. FCC, FCN, FCE and FCA affected expression of (A) proglucagon (GLP-1), (B) GIP and (C) PC1/3 genes. (D) Only FCA significantly affected cell viability. Cellular content of (E) GLP-1 and (F) GIP were also affected by plant extracts. Results are mean ± SEM (n=3). *P<0.05, **P<0.01 and ***P<0.001 compared with control, ns- not significant.

Figure 3. **Compounds isolated from *F. cretica* stimulate secretion of GLP-1 but not GIP.** QA, dQA and EdQA were isolated and identified from FCE. At a concentration of 50µM QA, dQA and EdQA all stimulated GLP-1 secretion (5.1-16.9-fold). EdQA also significantly increased GLP-1 secretion at 25µM. No comparable secretory effect for GIP was observed. Results are mean ± SEM (n=3). *P<0.05, **P<0.01 and ***P<0.001 compared with control, ns- not significant.

Figure 4. **Compounds isolated from *F. cretica* influence incretin hormone biosynthesis.** QA, dQA and EdQA affected the expression of (A) proglucagon (GLP-1), (B) GIP and (C) PC1/3 genes in STC-1 pGIP neo cells. Only EdQA significantly improved cell viability (D) whilst cell numbers (E) were unaffected by treatments. Improvements in
the cellular content were noted for GLP-1 (F) and GIP (G). Results are mean ± SEM (n=3). **P<0.01 and ***P<0.001 compared with control, ns- not significant.

**Figure 5. Compounds isolated from *F. cretica* activate the TGR5 receptor.** TGR5 receptor activation was determined by measurement of cAMP production in CHO cells stably expressing human TGR5 (GPBAR1). CHO QA, dQA and EdQA all activated TGR5 at a concentration of 100µM. EdQA also appeared to activate TGR5 at 33µM. Results are mean ± SEM (n=3). *P<0.05 and ***P<0.001 compared with control, ns- not significant.
Figure 1

A. GLP-1 Secretion (pM/10⁶ cells/h)

B. GLP-1 Secretion (pM/10⁶ cells/h)

C. GIP Secretion (pg/10⁶ cells/h)

D. GIP Secretion (pg/10⁶ cells/h)

125 µg/ml

250 µg/ml

Control FCC FCN FCE FCA

*** ns *** *** *** ***

Control FCC FCN FCE FCA

*** *** *** *** ***
Figure 2
Figure 3

A

GLP-1 Secretion (pM/10^6 cells/h)

Control
QA
dQA
EIAQA

25 µM

B

GLP-1 Secretion (pM/10^6 cells/h)

Control
QA
dQA
EIAQA

50 µM

C

GIP Secretion (pg/10^6 cells/h)

Control
QA
dQA
EIAQA

25 µM

D

GIP Secretion (pg/10^6 cells/h)

Control
QA
dQA
EIAQA

50 µM

ns

**
Figure 5

![Graph showing TGR5 agonism % increase (Control)](image)

- Control
- QA (33 μM, 100 μM)
- dQA (33 μM, 100 μM)
- EdQA (33 μM, 100 μM)

Significance:
- ns (not significant)
- * (p < 0.05)
- *** (p < 0.001)