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Modulation of satiety hormones by *Bacteroides thetaiotaomicron*, *Bacteroides fragilis and their* derivatives

Somayeh Vaezijoze[1], Shiva Irani[1], Seyed Davar Siadat[2,3*] and Mohammadreza Zali[4*]

Abstract

Obesity is a complex disorder influenced by various factors, including gut microbiota, which play a crucial role in metabolic regulation. This study is aimed to investigate the effects of *Bacteroides thetaiotaomicron* and *Bacteroides fragilis*, along with their derivatives—outer membrane vesicles (OMVs) and cell-free supernatant (CFS)—on the expression and secretion of satiety hormones in the murine intestinal secretin tumor cell line (STC-1). We examined the expression of peptide YY (*PYY*), glucagon-like peptide-1 and -2 (*GLP-1* and *GLP-2*, encoded by the *GCG* gene), the enzyme prohormone convertase-1 (*PC1/PCSK1* gene), and the receptors G protein-coupled receptor 119 and 120 (*GPR119* and *GPR120*), and G-protein-coupled bile acid receptor (*TGR5*). Our results demonstrate that live *B. fragilis* significantly increased *PYY* expression and secretion. *B. thetaiotaomicron* CFS notably upregulated *GCG*, *PCSK1*, *GPR119*, *GPR120*, and *TGR5* expression, leading to elevated *GLP-1* secretion. *B. fragilis* CFS decreased GPR119, GPR120, and GCG expression. OMVs from *B. thetaiotaomicron* at 50 µg/ml significantly enhanced *GCG* and *PCSK1* expression, while *B. fragilis* OMVs generally decreased gene expression, except for *PYY* protein abundance. Inactive *B. thetaiotaomicron* and *B. fragilis* increased *GCG* mRNA levels and *GLP-1* concentration, with inactive *B. fragilis* also elevating *GLP-2* protein levels.This study suggests that *B. thetaiotaomicron* and its derivatives, particularly CFS and OMVs, have potential as next-generation probiotics, postbiotics, and paraprobiotics for modulating satiety hormones and managing obesity. Further research is warranted to explore their mechanisms and therapeutic applications in vivo.

Keywords Obesity, Gastrointestinal microbiota, *Bacteroides thetaiotaomicron*, *Bacteroides fragilis*, Outer membrane vesicles (OMVs), Satiety hormones

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Introduction

Obesity is a complex disorder influenced by a combination of environmental, genetic, epigenetic, neurological, and endocrine factors, Bray et al. (2018), Di Vincenzo et al. 2024). It is characterized by low-grade inflammation, disruption of the gut barrier, and alterations in gut microbiota. Notably, an altered ratio of Firmicutes to Bacteroidetes is often observed in obese individuals, contributing to metabolic dysfunctions (Meehan et al. 2012; Ye et al. 2021; Tilg and Moschen 2024). *Bacteroides thetaiotaomicron* and *Bacteroides fragilis*, members of the Bacteroidetes family, are commensal Gram-negative anaerobic bacteria residing in the mammalian lower gut (Butler et al. 2023) B. *thetaiotaomicron* is known for its ability to regulate metabolism through glycosyl hydrolase enzymes that facilitate the digestion of polysaccharides, while, due to their beneficial interactions with the host, both bacteria are considered promising candidates for next-generation probiotics (Langella et al. 2019; Durant et al. 2020; Lu and Imlay 2021; Ye et al. 2021; Vasquez Ayala et al. 2024).

Recent research underscores the pivotal role of gut microbiota and their metabolites in influencing host metabolism, particularly through the modulation of satiety hormones (Martin et al. 2019). Enteroendocrine L cells in the gut mucosa synthesize and secrete key hormones such as peptide YY (PYY), glucagon-like peptide-1 (GLP-1), and glucagon-like peptide-2 (GLP-2). These hormones play crucial roles in regulating gut motility, satiety, and glucose metabolism. PYY enhances the feeling of fullness, reduces food intake, and modulates energy intake (Price and Bloom 2014). GLP-1, an incretin hormone, facilitates glucose homeostasis by stimulating insulin secretion, inhibiting glucagon secretion, and slowing gastric emptying, thereby promoting satiety and weight loss (Hoffman and Adeli 2024). GLP-2 supports intestinal epithelial proliferation, inhibits apoptosis, and reduces permeability, thus enhancing gut integrity and exerting anti-inflammatory effects (Fayfman et al. 2019). GLP-1 and GLP-2 are encoded by the common gene *GCG*, produced in L cells in the presence of prohormone convertase-1 (*PC1/PCSK1* gene) and tissue-specific post-translational processing (Ramos-Molina et al. 2016).

Enteroendocrine L cells express various G-protein-coupled receptors (GPCRs), such as TGR5, GPR119, and GPR120, which detect lumenal food and bacterial products and subsequently trigger the synthesis and secretion of satiety hormones (Covasa et al. 2019). TGR5 activation by bile acids promotes PYY and GLP-1 secretion and glucose homeostasis (Santos-Hernández et al. 2024). GPR119, expressed on pancreatic beta cells and intestinal K and L cells, influences glucose homeostasis, food absorption, and weight loss by stimulating insulin, GLP-1, GIP, and PYY transcription and secretion. Consequently, compounds regulating GPR119 expression are being explored for treating diabetes, obesity, and metabolic syndrome (Zhao et al. 2021). GPR120, a lipid-sensitive receptor, is involved in GLP-1 and GIP secretion, weight regulation, and anti-inflammatory responses (Xie et al. 2023).

Gut bacteria also interact with the host through biologically active molecules known as outer membrane vesicles (OMVs), which impact immunogenicity, inflammation, and the production of anti-inflammatory cytokines (Shen et al. 2012). Studies have shown that *B. thetaiotaomicron*, *B. fragilis*, and their OMVs do not exhibit toxic effects on human intestinal cells and can influence the gene expression of important cholesterol transporters (Badi et al. 2020a; Olovo et al. 2024).

In this study, we aim to extend the understanding of the interactions between gut microbiota and host metabolism by evaluating the in vitro effects of active and inactive *B. thetaiotaomicron* and *B. fragilis*, as well as their derivatives (OMVs and cell-free supernatant (CFS), on the expression and secretion of PYY, GLP-1, and GLP-2 in the STC-1 intestinal secretin tumor cell line (Kuhre et al. 2016; Yue et al. 2019; Kamakura et al. 2020). This cell line is routinely used to study gastrointestinal hormone secretion. Additionally, we will assess the mRNA levels of *Pcsk-1*, *Tgr5*, *Gpr119*, and *Gpr120* genes. By providing new comparative insights into the anti-obesity properties of *B. thetaiotaomicron*, *B. fragilis*, and their derivatives, this research may identify promising strategies for appetite management and obesity treatment.

Materials and methods

Bacterial strains and culture conditions

B. thetaiotaomicron CCUG 10,774 and *B. fragilis* ATCC 23,745 were obtained from the Pasteur Institute of Iran. The bacterial culture was performed in Brain Heart Infusion (BHI) broth (Quelab, Canada), as described previously. Briefly, the broth was supplemented with hemin (5 µg/ml), menadione (1 µg/ml), and 0.05% L-cysteine and incubated at 37 °C under anaerobic cultivation conditions (80% N2, 10% CO2, and 10% H2) using an Anoxomat™ MARK II system (Badi et al. 2020).

OMVs extraction

The OMVs were prepared as described previously (Claassen et al. 1996). Briefly, 12×10^8 colony-forming units (CFU/ml) of *B. thetaiotaomicron* and *B. fragilis* were cultured separately in 500 ml enriched BHI broth at 37 °C in anaerobic conditions overnight. Bacterial cells were harvested (5000 g, 4 °C, 30 min), washed twice with Phosphate-buffered Saline (PBS) solution, homogenized (sodium chloride 9%, 30 min), and concentrated (5000 g, 4 °C, 60 min). Next, the pellet was dispersed in Tris-ethylene diamine tetraacetic acid (EDTA)-sodium

deoxycholate (Sigma-Aldrich, USA) buffer and precipitated at 15,000 g for 90 min. After ultracentrifugation of the supernatant (130,000 g, 4 °C, 120 min), the pellet was suspended in sterile PBS. Finally, the OMVs suspensions were filtered through a 0.22-µm polyvinylidene difluoride filter (Millipore, Billerica, MA, USA) and stored at − 80 °C for subsequent use (Daliri et al. 2017; Badi et al. 2020a; Ghaderi et al. 2022).

Physicochemical characterization of OMVs

The physicochemical properties of extracted OMV*s* were determined by Transmission Electron Microscopy (TEM), spectrophotometry, and electrophoresis. The spectrophotometry method using a NanoDrop instrument at 280 nm was employed to measure the concentration of purified proteins (Badi et al. 2020). The protein profile was determined by 12% SDS-PAGE (Elhenawy et al. 2014).

Bacterial heat inactivation

B. thetaiotaomicron and *B. fragilis* were inactivated by heat. First, they were cultured in 100 ml enriched BHI broth medium (Hemin, L-Cysteine, and Menadione) and shaken for 22 h in 37 °C incubators. After washing the bacterial pellets, the resuspended bacteria were heat-killed at 70 °C for 30 min in a water bath and stored at − 80 °C until further use (Keshavarz Azizi Raftar, Ashrafian et al. 2021; Taddese et al. 2021).

Preparation of Cell-free supernatant (CFS)

As mentioned before, *B. thetaiotaomicron* and *B. fragilis* were cultured separately in 100 ml enriched BHI broth (Hemin, L-Cysteine, and Menadione) at 37°C for 24 h at 160 rpm. The bacterial CFS was prepared by the centrifugation of log-phase cultures of *B. thetaiotaomicron* (OD600~0.8) and *B. fragilis* (OD600~1) at 4,000 g and 4 °C for 10 min. Cell-free supernatants were filtered through a 0.22 µm polyethersulfone (PES) membrane filter, pH was adjusted at 7.4 and stored at − 80°C until use (Escamilla et al. 2012).

STC-1 culture

STC-1 cell line was obtained from Cell Bank of Belgium (ATCC®; CRL 3254). Cells were grown in high glucose Dulbecco's modified Eagle's Medium (DMEM, Cegrogen), supplemented with 17.5% Fetal Bovine Serum (FBS, Gibco™, MA, USA), 100 U/ml penicillin, 100 mg/l streptomycin, and 1% non-essential amino acids. The cells were incubated at 37 °C under 5% CO_2. Cells were passaged at 80–90% confluence (Verhoeckx et al. 2015).

Treatment of STC-1 cells with active and inactivated form of *B. thetaiotaomicron*, *B. fragilis*, and their derivatives

Cells were seeded at a density of 1.5×10^6 in each well of six-well plates and incubated in a 5% CO_2 humidified atmosphere at 37 °C for 24 h. In order to harmonize STC-1 cells growth, culture medium was replaced by serum and antibiotic-free DMEM for 4 h before treatment. Afterwards, for treatment of active and inactivated form of *B. thetaiotaomicron*, *B. fragilis* at the multiplicity of infection (MOI) both in 10 and 50 ratios (i.e., 10 and 50 bacteria per cell, respectively), OMVs (50 and 100 µg/ml), and CFS (25% v/v) were separately added to the wells and incubated for 2–3 h. The supernatant was collected by centrifugation (900 g for 5 min) to assess the hormone content. Finally, the cells of each well were harvested for further analysis (Verhoeckx et al. 2015).

Quantitative real-time polymerase chain reaction (qRT-PCR) analysis

The total RNA was isolated from treated STC-1 cells. 2000 ng of RNA was reverse-transcribed to complementary DNA (cDNA) YTA cDNA synthesis Kit, (cat No: YT4500) at 42 °C for 60 min. All primer sequences used in this study are shown in Table 1.

The qRT-PCR was performed by the SYBR Green method and Light Cycler® 96 SW 1.1 (Roche, Germany). A qPCR with 10 µl final volume was performed, 2X SYBR qPCR Master Mix, and specific primers (Table 1). The qPCR amplification condition included an initial denaturation for 60 d at 95 °C, followed by 40 cycles (denaturation: 95 °C, 10 s, annealing: 60 °C, 30 s, and extension at 72 °C, 30 s). RPL19 was selected as a housekeeping gene for comparing all targeted genes (for normalization).

Determination of PYY, GLP-1, and GLP-2 using ELISA

The total PYY, GLP-1, and GLP-2 proteins were measured from the cell culture supernatants using ZellBio Gmbh ENZYME-linked IMMUNOSORBENT ASSAY (ELISA) kit (Germany) according to the manufacturer's instructions, without using DPP-4 inhibitors. The satiety hormones assays were performed in triplicate.

Statistical analysis

All results were reported as means ± standard deviation of three independent experiments. The ΔΔCT method was used for the relative gene expression analysis, and RPL-19 was used as an internal control. The data extracted from qRT-PCR were analyzed by GraphPad Prism software (version 8.4.3; GraphPad Software Inc., San Diego, CA, USA) using an independent sample t-test (between two groups) and one-way Analysis of Variance (ANOVA), followed by Tukey's post hoc test to estimate differences between means which was used to compare means among more than two groups for each

Table 1 .List of primers

Primer name	Sequence	Primer size (bp)	Product size (bp)	References
m-*pyy*-forward	ACGGTCGCAATGCTGCTAAT	20	177	Abulaiti et al. (2017)
m-*pyy*-reverse	GACATCTCTTTTTCCATACCGCT	23		
m-*Tgr5*-forward	CTGTGTGAGATCCGCCGAC	19	176	Newman et al. (2020)
m-*Tgr5*-reverse	CGACGCTCATAGGCCAAGA	19		
m-*Gcg*-forward	CTTCCCAGAAGAAGTCGCCA	20	199	This study
m-*Gcg*-reverse	AGTGACTGGCACGAGATGTT	20		
m-*GPR119*-forward	GGTAACTGGCCAATCTGAAGACTA	24	223	This study
m-*GPR119*-reverse	GAGGTGATTCCAGACTGCTCT	21		
m-*GPR120*-forward	CTGGGGCTCATCTTTGTCGT	20	155	This study
m-*GPR120*-reverse	ACGACGAGCACTAGAGGGAT	20		
m-*PCSK1*-forward	TGTACTGCTTTCGCCTTCTTTT	22	84	This study
m-PCSK1-reverse	CGCCGCCCATTCATTAACA	19		
m-*RPL19*-forward	CCTGAAGGTCAAAGGGAATGTGTT	24	143	Bonomi et al. (2012)
m-*RPL19*-reverse	GCTTTCGTGCTTCCTTGGTCTTA	23		

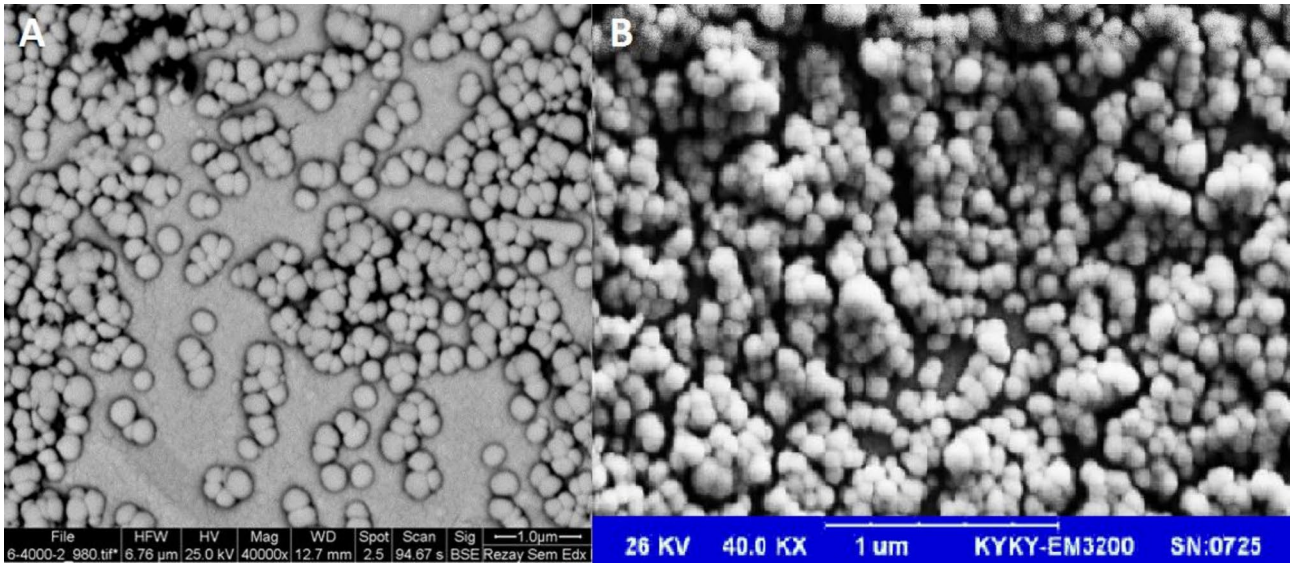

Fig. 1 The SEM images of OMVs which is extracted from **A***B. thetaiotaomicron* and **B***B. fragilis*

parameter. *P*-values less than 0.05 were considered statistically significant.

Results

Physicochemical characterization of OMVs

The TEM images revealed that the OMVs isolated from *B. thetaiotaomicron* and *B. fragilis* had spherical bilayer shapes with a mean size of 60–120 nm (Fig. 1).

The NanoDrop instrument results revealed that the total protein concentration of OMVs was about 0.4–1.9 mg/mL. The highest amount of protein was reported in the bands of 36, 47, and 75 kDa of *B.thetaiotaomicron*-derived OMVs and 25,45,60–63,and 50 kDa of *B.fragilis*-derived OMVs (Fig. 2).

Overview of treatments and effects on satiety hormone expression and secretion

To investigate the effects of *Bacteroides thetaiotaomicron* and *Bacteroides fragilis*, along with their derivatives, on the expression and secretion of satiety hormones, various conditions were tested. Table 2 summarizes the observed effects of different treatments on the STC-1 cell line.

Effects of *B. thetaiotaomicron* and *B. fragilis* on PYY, GCG, Pcsk1 and related receptors expression

The expression of PYY, GCG, and Pcsk1 in STC-1 cells treated with *B. thetaiotaomicron*, *B. fragilis* (MOI 10 and 50) was examined separately to investigate possible effects on satiety. According to qRT-PCR in Fig. 3A, B. *fragilis* at MOI 10 ($p < 0.001$) and MOI 50 ($p < 0.001$) upregulated the expression of PYY, while *B. thetaiotaomicron* at MOI 10 ($p = 0.026$) decreased the expression of this gene and

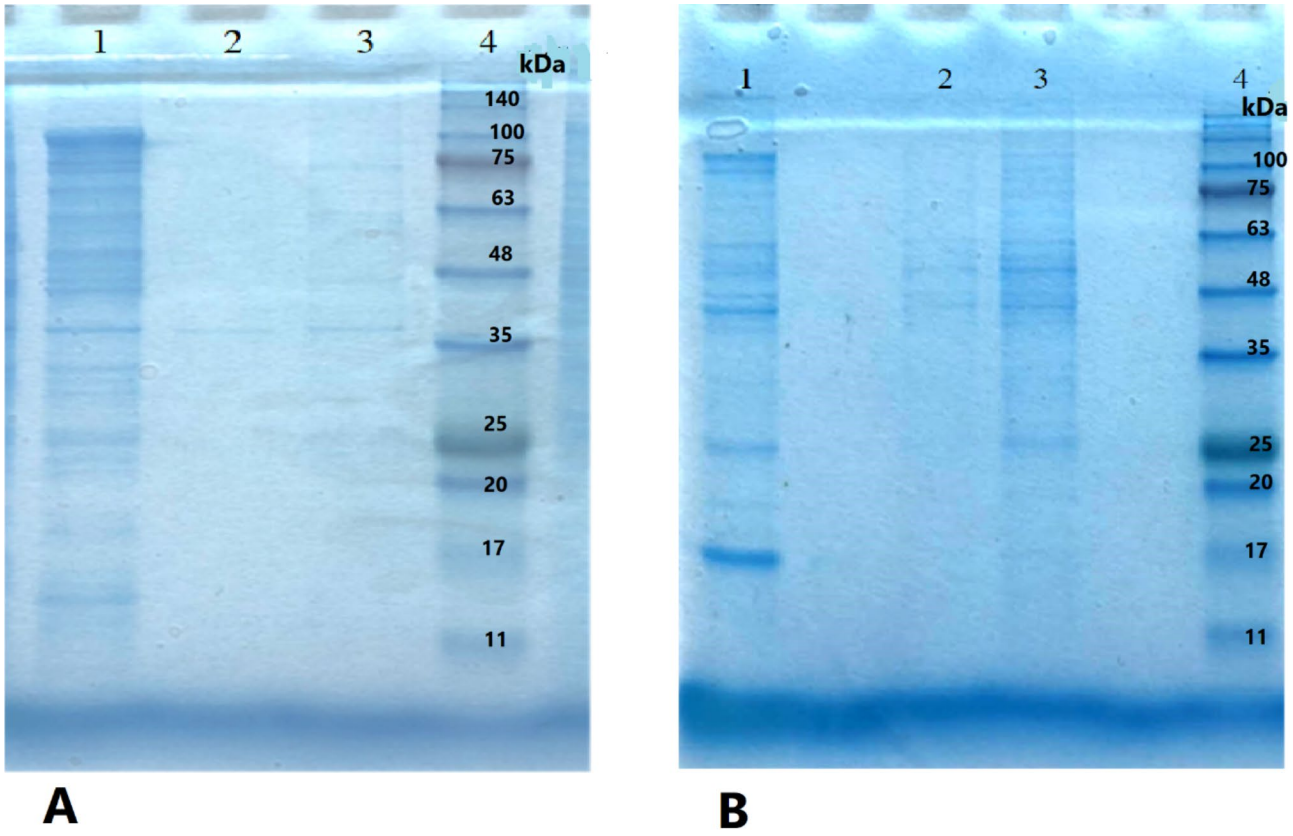

Fig. 2 Sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) showing the protein profile of **A***B. thetaiotaomicron* derived (1) Supernatant: 1.91 mg/ml, (2) OMV: 0. 43 mg/ml, (3) OMV: 0.79, (4): Ladder; **B***B. fragilis* derived (1) Supernatant: 1.72 mg/ml, (2) OMVs: 0.74 mg/ml, (3) OMVs: 0.9, (4): Ladder

Table 2 Summary of the effects of treatments on satiety hormone expression and secretion

Condition	Treatment	Concentration	Observed effects
Live bacteria	*Bacteroides thetaiotaomicron*	MOI 10	Decreased *Pyy* mRNA expression, increased PYY protein content, increased *Tgr5* and *Gpr120* mRNA expression
		MOI 50	Increased GLP-1 secretion
	Bacteroides fragilis	MOI 10	Increased *Pyy* mRNA and protein expression, decreased *Pcsk1* mRNA expression, increased *Gpr119* mRNA expression
		MOI 50	Increased PYY and GLP-1 secretion
Inactive bacteria	*Bacteroides thetaiotaomicron*	MOI 10	Increased *Gcg* mRNA expression, increased GLP-1 secretion
		MOI 50	Increased *Pyy*, *Gcg*, *Pcsk1*, *Gpr119*, and *Gpr120* mRNA expression, increased PYY and GLP-1 secretion
	Bacteroides fragilis	MOI 10	Increased *Tgr5* mRNA expression
		MOI 50	Increased *Pyy*, *Gcg*, and *Tgr5* mRNA expression, increased PYY, GLP-1, and GLP-2 secretion
OMVs	*Bacteroides thetaiotaomicron* OMVs	50 µg/ml	Increased *Gcg* and *Pcsk1* mRNA expression, increased PYY and GLP-1 secretion
		100 µg/ml	Decreased *Tgr5* and *Gpr119* mRNA expression
	Bacteroides fragilis OMVs	50 µg/ml	Decreased *Pcsk1* and *Gpr119* mRNA expression
		100 µg/ml	Decreased *Gcg*, *Tgr5*, and *Gpr120* mRNA expression
CFS	*Bacteroides thetaiotaomicron* CFS	25% v/v	Increased *Gcg*, *Gpr119*, *Gpr120*, and *Tgr5* mRNA expression, increased GLP-1 secretion, decreased PYY secretion
	Bacteroides fragilis CFS	25% v/v	Decreased *Gcg*, *Gpr119*, and *Gpr120* mRNA expression, decreased GLP-1 secretion

the result was not significant for MOI 50. As shown in Fig. 3A, MOI 10 and MOI 50 of *B. fragilis* ($p = 0.0025$, $p = 0.0185$, respectively) could significantly decrease the mRNA level of PCSK1 but *B.thetaiotaomicron* could not. Neither *B. thetaiotaomicron* nor *B. fragilis* cause any significant changes in the expression of *Gcg* gene.

As seen in Fig. 3A, B. *thetaiotaomicron* MOI 10 and 50 significantly enhanced the expression of TGR5 ($p < 0.0001$), and at MOI 10 the mRNA expression of

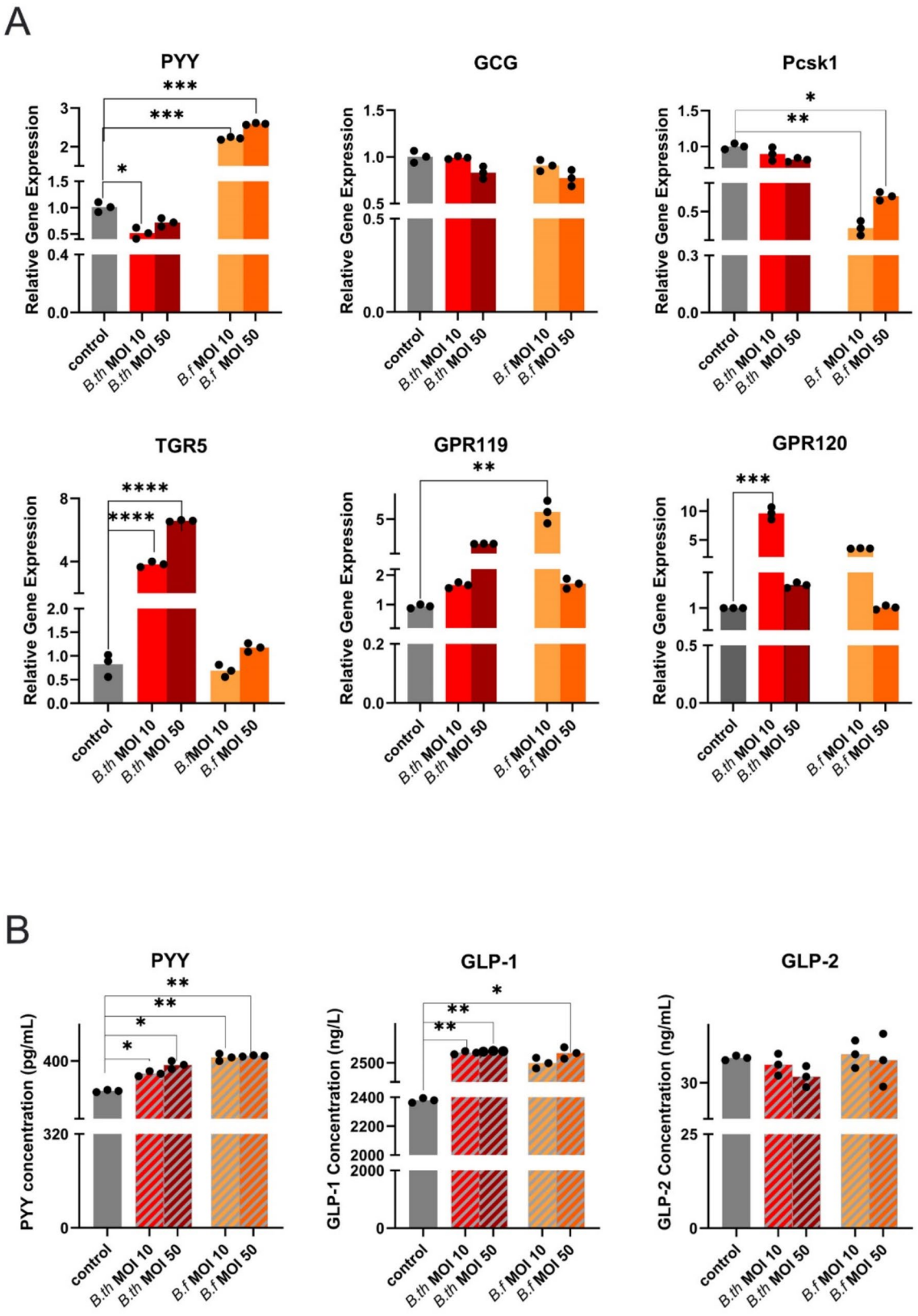

Fig. 3 **A** The effect of *B. thetaiotaomicron*, *B. fragilis* (MOI 10 and MOI 50) on *Pyy*, *Pcsk1*, *Gcg*, *Tgr5*,*Gpr119* and *Gpr120* mRNA expression for 2–3 h in STC-1 cells. **B** The effect of *B. thetaiotaomicron*, *B. fragilis* (MOI 10 and MOI 50) on PYY (pg/mL), GLP-1(ng/mL), GLP-2 (ng/mL) levels for 2–3 h in STC-1 cells measured by ELISA. Significancy is evaluated in comparison with control. Data are shown as the mean ± SD. (*) represent significant changes, respectively $*p < 0.05$, $**p < 0.01$,$***p < 0.001$, and $****p < 0.0001$ by one-way ANOVA and t-test statistical analysis. MOI: multiplicity of infection

GPR120 ($p = 0.0003$) was increased, but did not affect GPR119 transcription in comparison with control group. However, *B. fragilis* did not have any significant effect on the TGR5 and GPR120 genes, while GPR119 mRNA level ($p = 0.003$) was increased in response to treatment with *B. fragilis* at MOI 10.

We used ELISA kit to analyze PYY, GLP-1, and GLP-2 level in response to *B. thetaiotaomicron*, *B. fragilis* treatments. Expression data (Fig. 3B) showed the significant elevation of PYY at MOI 10($p = 0.024$) and GLP-1 at both MOIs ($p = 0.0026$, $p = 0.0023$) of *B. thetaiotaomicron*. levels of PYY ($p = 0.0009$) and GLP-1 ($p = 0.035$) at MOI 50 of *B. fragilis* were significantly increased. While, the level of GLP-2 did not change significantly by any types of bacterial treatments.

Impacts of inactive *B. thetaiotaomicron*, *B. fragilis*, on PYY, GCG, Pcsk1 and related receptors expression

To investigate whether inactive *B. thetaiotaomicron*, inactive *B. fragilis* might reduce obesity, we assessed the expression of gut hormones at the mRNA and protein levels and receptor genes involved in GLP-1 and GLP-2 secretion. For this purpose, STC-1 cells were treated with inactive *B. thetaiotaomicron* (MOI 10 and 50). As demonstrated in Fig. 4A, at MOI 50, following qRT-PCR, PYY ($p = 0.0026$), GCG ($p = 0.0017$), PCSK1 ($p = 0.0095$), GPR119 ($p = 0.029$), and GPR120 ($p = 0.013$) transcript were increased; the only significant effect of inactive *B.thetaiotaomicron* at MOI 10 was observed on the enhancement of GCG ($p = 0.0024$) transcription. The inactivated form of the bacterium had no significant effect on TGR5 mRNA expression in the STC-1 cell line. We also evaluated PYY, GLP-1, and GLP-2 secretion. The ELISA data showed that PYY level was increased in exposure to MOI 50 of inactivated form of *B. thetaiotaomicron* ($p = 0.021$), while MOI 10 had no significant effect on it. Also secretion level of GLP-1 was increased at both MOI 10 and 50 ($p = 0.0158$, $p = 0.0046$, respectively). No significant change was observed in GLP-2 secretion level (Fig. 4B).

Treatment of STC-1 cells with inactive *B. fragilis* (MOI 10 and 50) showed that at MOI 50 significantly the expression of PYY *($p = 0.0013$)*, *GCG ($p = 0.0004$)*, and *TGR5* ($p = 0.024$) in mRNA level were increased. The only significant effect of inactive *B. fragilis* at MOI 10 was on TGR5 ($p = 0.0122$) gene, which increased its transcription. The expression of genes PCSK1, GPR119, GPR120 did not show significant changes. The ELISA data showed the significant effect of inactive *B. fragilis* on PYY ($p < 0.01$), GLP-1 ($p < 0.01$), and GLP-2 ($p < 0.05$) in protein level. As seen in Fig. 4B, all three gut hormone genes responded positively to this treatment.

Effects of *B. thetaiotaomicron* and *B. fragilis* derived OMVs on the satiety relevant genes

We treated STC-1 cells with *B. thetaiotaomicron* and *B. fragilis* derived OMVs at 50 and 100 mg/ml. As demonstrated in Fig. 5A, B. *thetaiotaomicron* OMVs at 50 µg/ml significantly enhanced GCG ($p < 0.0001$), and PCSK1 ($p = 0.023$) mRNA expression; However, *B. thetaiotaomicron* OMVs at 100 µg/ml had no significant impact on the given genes. The mRNA expression of TGR5 ($p = 0.022$) and GPR119 ($p = 0.084$) receptors were significantly reduced by *B. thetaiotaomicron* OMVs 100 µg/ml treatment. PYY and GPR120 mRNA reduction was not significant when exposed to *B. thetaiotaomicron* derived OMVs at both concentrations. Beside, the significant decreasing effect of *B. fragilis* derived OMVs at 100 µg/ml on mRNA expression of GCG ($p = 0.0414$), TGR5 ($p = 0.0133$)and at 50 µg/ml on Pcsk1 ($p = 0.035$), GPR119 ($p = 0,0211$) were shown in Fig. 5A. *B.fragilis* OMV reduced GPR120 transcription significantly; while it did not cause any significant effect on PYY gene expression.

The ELISA data showed a significant elevation in level of PYY and GLP-1 ($p = 0.0052$, $p = 0.0063$ respectively) in response to *B. thetaiotaomicron* derived- OMV 50 µg/ml (Fig. 5B); However, there were no significant changes in GLP-2 level. As seen in Fig. 5B, Levels of GLP-1 were significantly decreased ($p = 0.0361$) when exposed to *B. fragilis*-derived OMVs, while the levels of PYY and GLP-2 did not show significant changes.

Effects of CFS of *B. thetaiotaomicron* and *B. fragilis* on genes related to obesity

We incubated STC-1 cells with *B. thetaiotaomicron* and *B. fragilis* CFS for 2–3 h separately. The results of qRT-PCR are shown in Fig. 6A. *B. thetaiotaomicron* CFS significantly induced the expression of *GCG* ($p = 0.0135$), *GPR119* ($p = 0.0066$), *GPR120* ($p = 0.0048$), and *TGR5* ($p = 0.0139$), but made no significant change in *PYY* and *PCSK1* mRNA transcription. In contrast, our results showed that *B. fragilis* CFS decreased the expression of *GCG* ($p = 0.033$), *GPR119* ($p = 0.046$), and *GPR120* ($p = 0.037$) but made no significant changes in *PYY*, *PCSK1*, and *TGR5* mRNA expression.

Moreover, ELISA results also showed a significant increase in the levels of GLP-1 ($p = 0.026$), a significant decrease in PYY levels ($p = 0.044$), and no significant change in GLP-2 abundance in *B. thetaiotaomicron* CFS treatment. GLP-1 ($p = 0.0423$) secretion levels decreased significantly under *B. fragilis* CFS exposure, while levels of PYY and GLP-2 did not show significant changes (Fig. 6B).

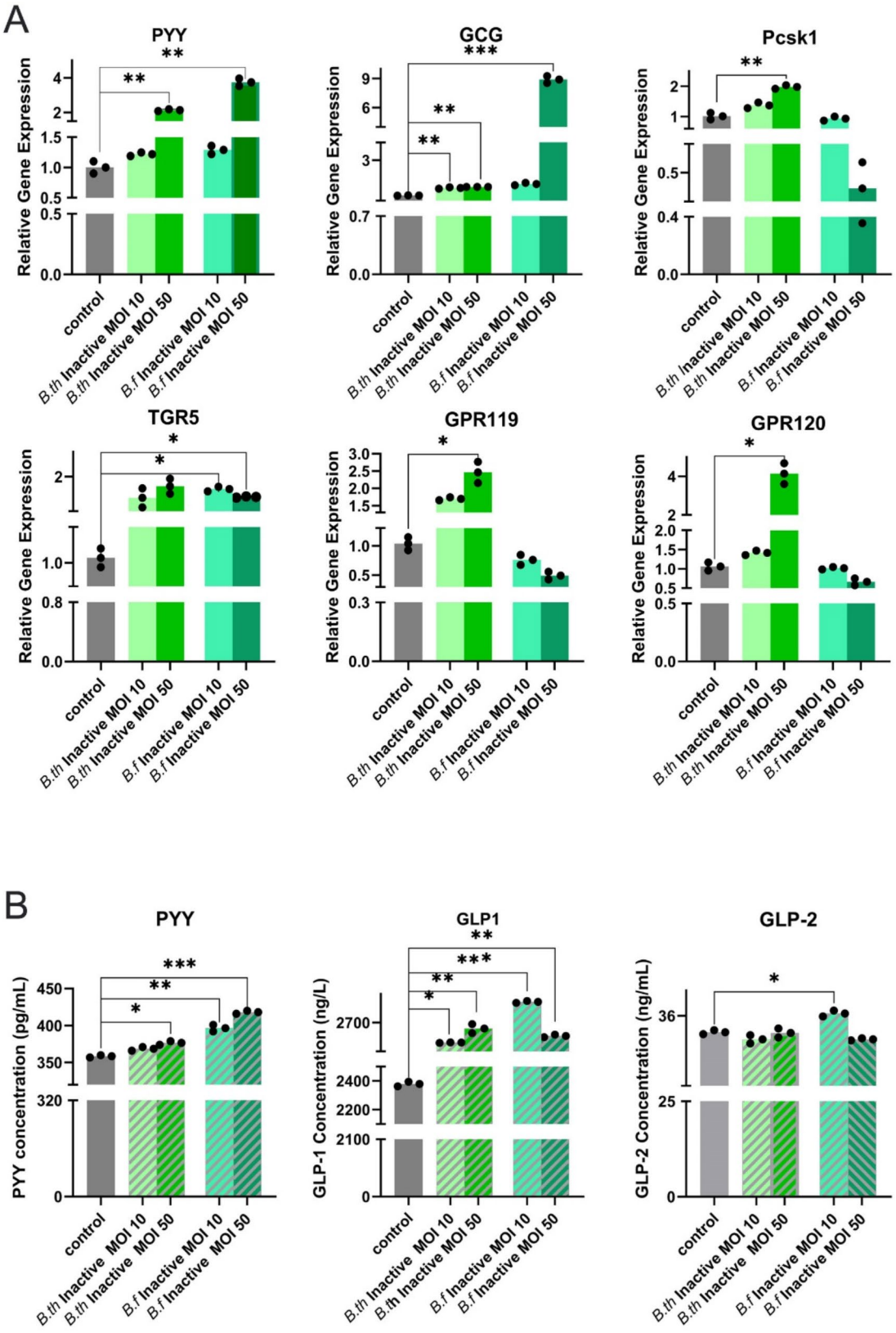

Fig. 4 **A** The effect of inactive *B. thetaiotaomicron*, inactive *B. fragilis* (MOI 10 and MOI 50) on *Pyy*, *Pcsk1*, *Gcg*, *Tgr5*,*Gpr119* and *Gpr120* mRNA expression for 2–3 h in STC-1 cells. **B** The effect of inactive *B. thetaiotaomicron*, inactive *B. fragilis* (MOI 10 and MOI 50) on PYY (pg/mL), GLP-1 (ng/mL), GLP-2(ng/mL) levels for 2–3 h in STC-1 cells measured by ELISA. Significancy is evaluated in comparison with control. Data are shown as the mean ± SD. (*) represent significant changes, respectively $*p < 0.05$, $**p < 0.01$,$***p < 0.001$, and $****p < 0.0001$ by one-way ANOVA and t-test statistical analysis. MOI: multiplicity of infection

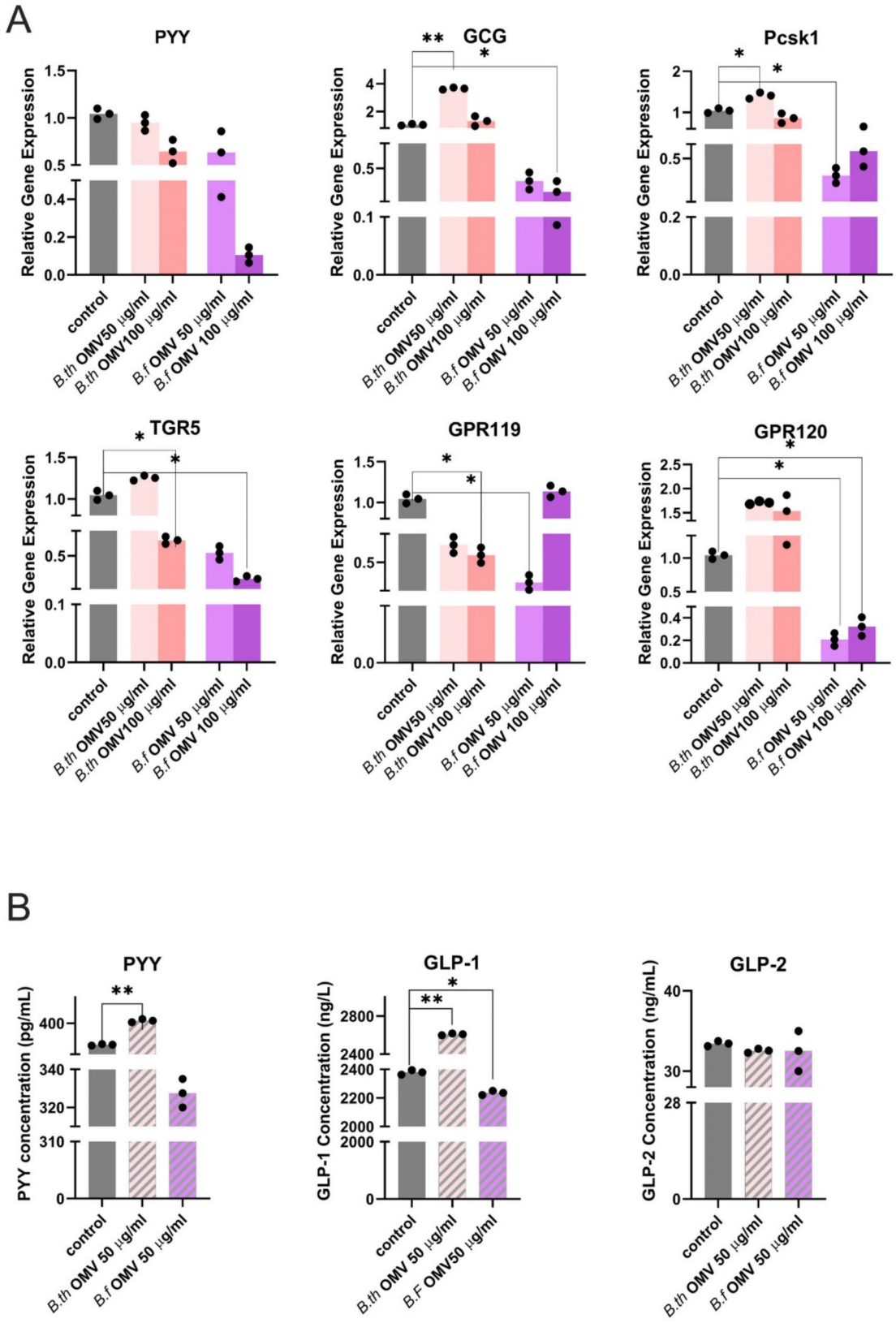

Fig. 5 **A** The effect of *B. thetaiotaomicron*, *B, fragilis* derived OMVs (50 and 100 µg/ml) on *Pyy*, *Pcsk1*, *Gcg*, *Tgr5*, *Gpr119* and *Gpr120* mRNA expression for 2–3 h in STC-1 cells. **B** The effect of *B. thetaiotaomicron*, *B. fragilis* derived OMVs (50 and 100 µg/ml) on PYY (pg/mL), GLP-1 (ng/mL), GLP-2 (ng/mL) levels for 2–3 h in STC-1 cells measured by ELISA. Significancy is evaluated in comparison with control. Data are shown as the mean ± SD. (*) represent significant changes, respectively $*p < 0.05$, $**p < 0.01$,$***p < 0.001$, and $****p < 0.0001$ by one-way ANOVA and t-test statistical analysis

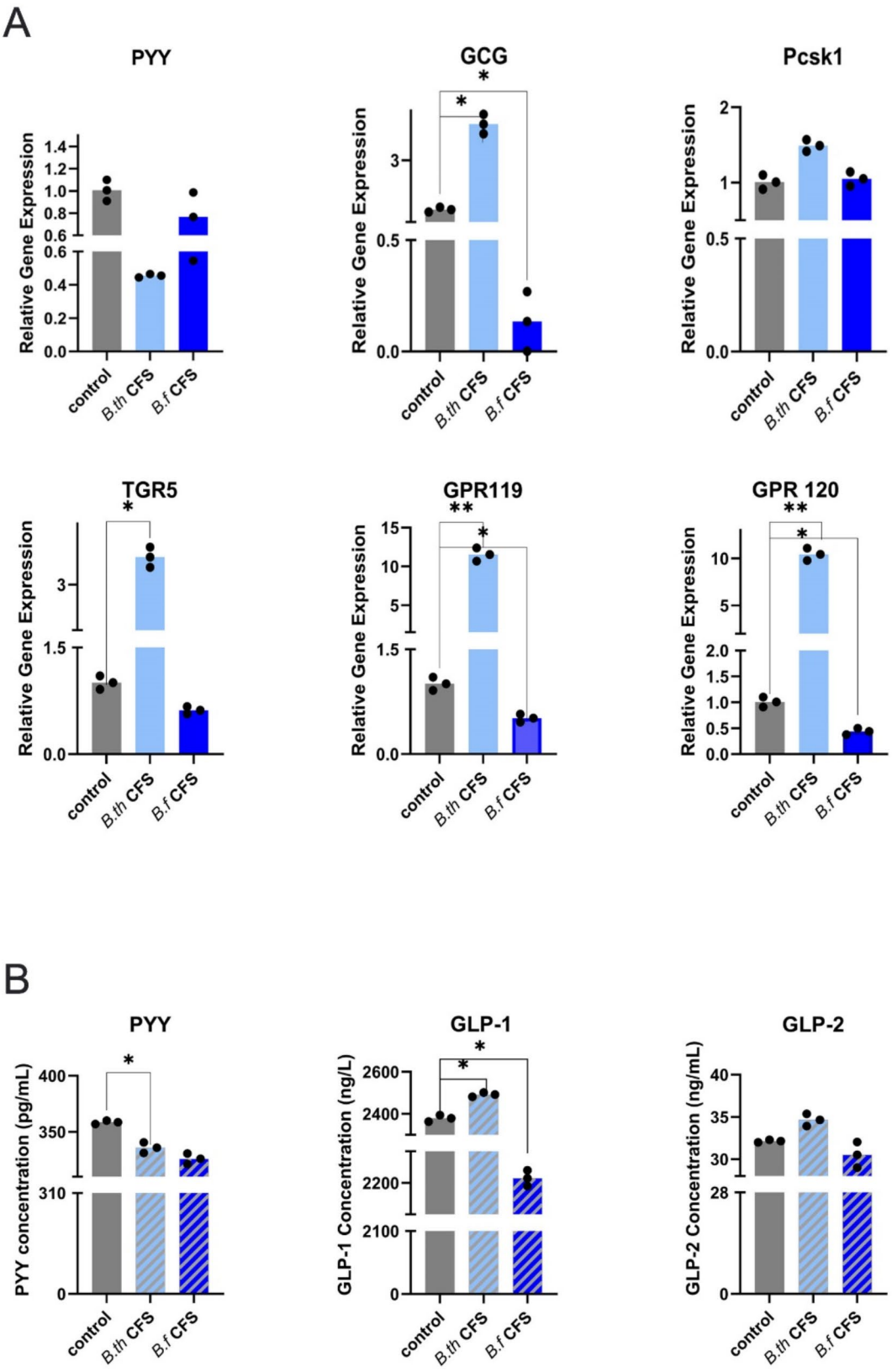

Fig. 6 **A** The effect of *B. thetaiotaomicron*, *B. fragilis* CFS (25% V/V) on *Pyy*, *Pcsk1*, *Gcg*, *Tgr5*, *Gpr119* and *Gpr120* mRNA expression for 2–3 h in STC-1 cells. **B** The effect of *B. thetaiotaomicron*, *B. fragilis* CFS (25% V/V) on PYY(pg/mL), GLP-1 (ng/mL), GLP-2 (ng/mL) levels, for 2–3 h in STC-1 cells measured by ELISA. Significancy is evaluated in comparison with control. Data are shown as the mean ± SD. (*) represent significant changes, respectively *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$ by one-way ANOVA and t-test statistical analysis

Discussion

The gut microbiota has emerged as a crucial target for the prevention and treatment of metabolic disorders such as obesity and inflammatory bowel disease (IBD) (Davis 2016; Zuo and Ng 2018). Intestinal microbiota and their metabolites can affect metabolism by influencing the secretion of satiety hormones like PYY, GLP-1, and GLP-2, which play vital roles in regulating appetite, glucose homeostasis, metabolic balance, and gut integrity. Probiotics, as food supplements, have shown beneficial health effects by improving digestion, reducing dysbiosis, modulating the host immune response, and alleviating symptoms of certain diseases (Behbahani et al. 2019; Plaza-Diaz et al. 2019; Barzegar et al. 2021). This study delves into the effects of *Bacteroides thetaiotaomicron* and *Bacteroides fragilis*, alongside their derivatives—outer membrane vesicles (OMVs) and cell-free supernatant (CFS)—on the expression and secretion of key satiety hormones in the STC-1 intestinal cell line.

Our findings indicate that live *B. fragilis* significantly increases both the mRNA and protein levels of peptide YY (PYY), suggesting its potential to enhance satiety signals. Conversely, live *B. thetaiotaomicron* exhibited a decrease in *Pyy* mRNA expression but an increase in PYY protein levels, highlighting a complex post-transcriptional regulatory mechanism that necessitates further exploration.

The results from *B. thetaiotaomicron* CFS were particularly noteworthy. This treatment significantly upregulated the expression of glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2), prohormone convertase-1 (PC1), and the receptors GPR119, GPR120, and TGR5. This upregulation led to increased GLP-1 secretion, emphasizing the potential of *B. thetaiotaomicron* CFS in enhancing gut hormone signaling pathways related to glucose homeostasis and satiety. On the other hand, *B. fragilis* CFS decreased the expression of these receptors and GLP-1 mRNA, indicating a distinct regulatory effect compared to *B. thetaiotaomicron*.

OMVs from *B. thetaiotaomicron* at a concentration of 50 µg/ml significantly enhanced the expression of GLP-1 production and secretion genes. The corresponding increase in GLP-1 protein levels suggests that *B. thetaiotaomicron* OMVs can modulate enteroendocrine functions effectively. However, at a higher concentration of 100 µg/ml, there was a reduction in gene expression, likely due to the inhibitory effects of high concentrations of microbe-associated molecular patterns (MAMPs) and lipopolysaccharides (Elhenawy et al. 2014; Taşçı and Bingöl 2018).

Inactive forms of *B. thetaiotaomicron* and *B. fragilis* also demonstrated significant effects on satiety hormone expression. Inactive *B. thetaiotaomicron* increased the expression of several key metabolic genes, indicating that non-viable bacteria can still modulate metabolic pathways through their structural components and residual metabolic products. Inactive *B. fragilis* notably elevated GLP-2 protein levels, suggesting its role in maintaining gut integrity and anti-inflammatory responses. Studies indicate that inactive bacteria can play a better role in leaky gut conditions without multiplying and spreading in underlying tissues. For instance, Plovier's findings showed that pasteurized *Akkermansia muciniphila* promoted metabolism in obese and diabetic mice, and Ashrafian et al. indicated that pasteurized *A. muciniphila* had a more remarkable effect on obesity than its active form (Plovier et al. 2017; Ashrafian et al. 2021).

The differential effects observed between active and inactive bacteria, as well as their derivatives, highlight the complexity of host-microbiota interactions. The ability of *B. thetaiotaomicron* derivatives, particularly CFS and OMVs, to upregulate satiety-related genes and hormones underscores their potential as next-generation probiotics, postbiotics, and paraprobiotics. These findings pave the way for developing microbial-based interventions for managing obesity and related metabolic disorders.

The expression of G-protein-coupled receptors (GPCRs) such as TGR5, GPR119, and GPR120 plays a pivotal role in the regulation of satiety hormones and obesity (Patil et al. 2024).TGR5 activation by bile acids promotes PYY and GLP-1 secretion, contributing to glucose homeostasis (Wang et al. 2023). GPR119 and GPR120, sensitive to lipids, influence glucose homeostasis, weight regulation, and anti-inflammatory responses (Yang et al. 2024). Our study shows that *B. thetaiotaomicron* and its derivatives can significantly modulate these receptors, enhancing the secretion of satiety hormones and providing a promising avenue for therapeutic interventions.

Further in vivo studies are needed to confirm these findings and explore the underlying mechanisms in greater detail. The promising results from this in vitro study suggest that targeting gut microbiota and their metabolites could be a viable strategy for appetite management and obesity treatment.

This study highlighted the significant role of gut microbiota, specifically *Bacteroides thetaiotaomicron* and *Bacteroides fragilis*, in modulating satiety hormones and related gene expressions, which are crucial for managing obesity. Our findings demonstrate that *B. thetaiotaomicron* and its derivatives, particularly cell-free supernatant (CFS) and outer membrane vesicles (OMVs), significantly upregulate the expression of key satiety hormones such as GLP-1 and GLP-2, and enhance the expression of receptors involved in their secretion, including GPR119, GPR120, and TGR5. This suggests their potential as next-generation probiotics, postbiotics, and paraprobiotics for

therapeutic interventions in obesity and related metabolic disorders.

In contrast, *B. fragilis* exhibited a more variable influence, with its live form significantly increasing PYY expression and secretion, while its CFS generally reduced the expression of key genes associated with satiety. The differential effects between active and inactive forms of these bacteria highlight the complexity of their interactions with host metabolic pathways.

The ability of *B. thetaiotaomicron* derivatives to modulate enteroendocrine functions presents a promising avenue for developing microbial-based treatments targeting metabolic health. Further in vivo studies are necessary to validate these in vitro findings and elucidate the precise mechanisms by which these bacteria and their derivatives influence metabolic processes.

In summary, our research provides new insights into the beneficial roles of specific gut microbiota and their derivatives in regulating satiety hormones, offering potential strategies for appetite management and obesity treatment. The findings support the continued exploration of gut microbiota as therapeutic targets for metabolic health interventions.

Authors' contributions
S.V.J: Conceptualization, methodology, validation and writing; S.I., formal analysis, and data. curation; M.Z and S.D.S: review and editing, supervision, and project administration. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials
The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All authors have consented to the publication of this research.

Competing interests
The authors declare no conflicts of interest.

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