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Evaluation of Antidiabetic Compounds in Differentiated Adipocytes

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Abstract
Background:

Adipose tissue plays an important role in regulating glucose and lipid metabolism and maintaining insulin sensitivity. Apart from lowering blood glucose levels, antidiabetic drugs may also directly affect adipocyte function. The present study was conducted to compare the effects of common anti-diabetics, metformin, dapagliflozin, sitagliptin, pioglitazone, and glimepiride on 3T3-L1 derived adipocytes. Gene expression of key players involved in adipogenesis, lipid metabolism, adipokine production and inflammation was analyzed using quantitative real-time PCR. Each drug produced a distinct pattern of gene expression of these selected markers. Sitagliptin showed the strongest increase in the expression of adipogenic and lipid metabolism-related genes, suggesting improved adipocyte function. Pioglitazone markedly increased adiponectin expression with reduced inflammatory gene expression, indicating enhanced insulin sensitivity and adipocyte function. Glimepiride moderately increased the expression of adipogenic genes while reducing TNF-α expression, suggesting mild anti-inflammatory effects. Metformin caused only minor changes in most genes, with a noticeable reduction in PPARγ expression. Dapagliflozin increased the expression of genes involved in fatty acid uptake and lipid storage but also increased TNF-α expression. Overall, the findings demonstrate that different antidiabetic drugs have distinct effects on adipocyte gene expression, highlighting their diverse roles in regulating adipocyte metabolism and function beyond their glucose-lowering effects.

Key words: Diabetes type 2, Metformin, Glimepiride, Sitagliptin, Pioglitazone, Dapagliflozin.

Introduction

Adipose tissue is an important metabolic and endocrine organ that plays a central role in the regulation of energy homeostasis, glucose metabolism, lipid storage, and systemic insulin sensitivity (Coelho et al., 2013). It functions as a reservoir for energy in the form of triglycerides. Adipose tissue secretes numerous bioactive molecules, collectively known as adipokines, including adiponectin, leptin, resistin, and TNF-α, which regulate metabolic and immune functions (Kershaw & Flier, 2004). In obesity and type 2 diabetes mellitus (T2DM), adipose tissue undergoes profound structural and functional alterations characterized by adipocyte hypertrophy, dysregulated lipid metabolism, chronic low-grade inflammation, and insulin resistance. These pathological changes contribute to the development and progression of metabolic disorders (Guilherme et al., 2008).

The murine 3T3-L1 preadipocyte cell line is one of the most extensively characterized and widely used in vitro models for studying adipose physiology. This includes adipocyte differentiation, lipid metabolism, insulin signaling, and adipokine secretion. Upon proper stimulation, 3T3-L1 preadipocytes differentiate into mature adipocytes that exhibit many characteristics of white adipose tissue such as lipid droplet accumulation, insulin-responsive glucose uptake. The differentiated adipocytes also show expression of adipocyte-specific genes such as PPAR-γ, CEBP-α, AdipoQ, FASN, CD36, FATP1, and PLIN2 (Farmer, 2006). Therefore, differentiated 3T3-L1 adipocytes are considered as a robust system for evaluating the direct cellular effects of antidiabetic agents on adipocyte metabolism independent of other systemic physiological influences.

Although antidiabetic drugs are primarily prescribed to improve glycaemic control, accumulating evidence demonstrates that many of these agents exert pleiotropic effects on adipose tissue beyond glucose lowering (LaMoia & Shulman, 2021; Soccio et al., 2014). These effects include modulation of adipocyte differentiation, lipid uptake and storage, mitochondrial function, insulin sensitivity, oxidative stress, inflammatory signaling, and adipokine secretion. Therefore, understanding these direct actions is essential for elucidating the mechanisms by which antidiabetic therapies improve metabolic homeostasis and minimise associated adverse effects.

Metformin, a first-line therapy for T2DM, primarily exerts its metabolic effects through activation of AMP-activated protein kinase (AMPK). AMPK is a key regulator of cellular energy balance. AMPK activation suppresses hepatic gluconeogenesis and increases glucose utilization and fatty acid oxidation (Zhou et al., 2001). Metformin has been shown to improve insulin sensitivity, reduce lipogenesis, inhibit excessive lipid accumulation, enhance mitochondrial function, and suppress inflammatory pathways, partly through inhibition of nuclear factor-kappa B (NF-κB) signaling (Viollet et al., 2012). Metformin is also known to reduce oxidative stress and increase adiponectin production (Zulian et al., 2011).

Dapagliflozin, a selective sodium-glucose cotransporter-2 (SGLT2) inhibitor, lowers blood glucose by promoting urinary glucose excretion. It improves adipose tissue metabolism by reducing oxidative stress, reducing inflammatory responses while promoting fatty acid oxidation, and enhancing mitochondrial function. Studies have also reported improvements in adipocyte insulin sensitivity and reductions in adipocyte hypertrophy (Díaz-Rodríguez et al., 2018; Op den Kamp et al., 2021).

Sitagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor, prolongs the activity of endogenous incretin hormones, particularly glucagon-like peptide-1 (GLP-1) thus, enhancing glucose-dependent insulin secretion. Emerging evidence indicates that sitagliptin also exerts anti-inflammatory and antioxidant effects within adipose tissue (Dobrian et al., 2011). It has been reported to improve adipokine profiles, enhance insulin sensitivity, reduce inflammatory cytokine production, and oxidative stress, collectively contributing to improved adipocyte function (Rabe et al., 2008).

Pioglitazone, a member of the thiazolidinedione class, is a potent agonist of peroxisome proliferator-activated receptor gamma (PPARγ), the master transcriptional regulator of adipogenesis. Activation of PPARγ promotes adipocyte differentiation, enhances lipid storage in subcutaneous adipose tissue, increases adiponectin secretion, and improves systemic insulin sensitivity (Lehmann et al., 1995). Pioglitazone also suppresses inflammatory gene expression and promotes redistribution of lipids away

from ectopic tissues, reducing lipotoxicity and improving metabolic homeostasis. Even though it sometimes increases adiposity, pioglitazone generally enhances adipocyte functionality and insulin responsiveness (Soccio et al., 2014).

Glimepiride, a second-generation sulfonylurea, lowers blood glucose primarily by stimulating insulin secretion from pancreatic β-cells by inhibiting of ATP-sensitive potassium channels. Although its principal mechanism involves pancreatic action, accumulating studies suggest that glimepiride may exert direct effects on peripheral tissues, including adipocytes such as improvement in insulin-mediated glucose uptake, modulation of lipid metabolism, attenuation of oxidative stress and reduction of inflammatory signaling(Müller & Geisen, 1996). However, its direct effects on adipocyte differentiation and metabolic gene expression remain less extensively characterized compared with metformin and pioglitazone.

As these antidiabetic agents exert their therapeutic effects through different molecular pathways, comparative investigation of their direct actions on adipocytes is important for understanding their differential effects on adipose tissue function and biology. Analysis of adipogenic transcription factors (PPAR-γ and CEBP-α), lipid metabolism-related genes (FASN, CD36, FATP1, and PLIN2), adipokines (AdipoQ and RBP4), and inflammatory mediators such as TNF-α provides valuable insights into how these drugs affect adipocyte differentiation, lipid handling, endocrine function, and inflammatory status.

Therefore, the present study aims to investigate and compare the effects of metformin, dapagliflozin, sitagliptin, pioglitazone and glimepiride on differentiated 3T3-L1 adipocytes by evaluating changes in adipogenic markers, lipid metabolism-associated genes, adipokines expression and inflammatory mediators. A comprehensive understanding of these drug-specific effects will provide important insights into the immunometabolic actions of antidiabetic therapies and may contribute to the development of more targeted strategies for the treatment of obesity-associated insulin resistance and type 2 diabetes mellitus.

Table 1: Oral anti-diabetic agents used in this study

Drug	Drug Class	Mechanism of Action
Metformin	Biguanide	Activates AMP-activated protein kinase (AMPK)
Glimepiride	Sulfonylurea	Blocks ATP-sensitive potassium (K_{ATP}) channels
Sitagliptin	DPP-4 (Dipeptidyl Peptidase-4) Inhibitor	Inhibits DPP-4 enzyme, enhancing incretin activity
Pioglitazone	Thiazolidinedione	Activates peroxisome proliferator-activated receptor gamma (PPARγ)
Dapagliflozin	SGLT-2 (Sodium-Glucose Cotransporter-2) Inhibitor	Inhibits renal glucose reabsorption by blocking SGLT-2

Materials and Methods

Adipocyte differentiation and treatment

The murine embryonic fibroblast cell line 3T3-L1 was employed for adipocyte differentiation. Cells were seeded into 6-well plates at a density of 4×10^4 cells per well and cultured for 48 h. Subsequently, differentiation was induced using a differentiation medium consisting of 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), 1.0 µM dexamethasone, and 1.0 µg/mL bovine insulin prepared in basal medium (DMEM supplemented with 10% FBS). Cells were maintained under standard culture conditions (37°C, 5% CO_2) for 48 h. Following the induction period, the differentiation medium was replaced with adipocyte maintenance medium comprising DMEM supplemented with 10% FBS and 1.0 µg/mL bovine insulin to support adipocyte maturation and maintenance. Eight days post induction treatment was done with the drugs for 24 hrs before harvesting cells.

Drugs concentration used were as follows; metformin 1 mM, glimepiride 30 nM, sitagliptin 200 nM,

pioglitazone 10 µM and dapagliflozin 30 nM.

Gene Expression Analysis

Total RNA was isolated from treated and untreated cells using an RNA extraction reagent according to the manufacturer's instructions (RNeasy Mini Kit, Catalog No. 74104 QIAGEN). The concentration and purity of the extracted RNA were determined spectrophotometrically by measuring absorbance at 260 and 280 nm, and RNA integrity was verified prior to downstream analysis. One microgram of total RNA was reverse transcribed into cDNA using a commercially available reverse transcription kit (Applied Biosystems™ High-Capacity cDNA Reverse Transcription Kit, Cat. No. 4368814) following the manufacturer's protocol.

Quantitative real-time polymerase chain reaction was performed using a SYBR Green-based PCR Master Mix in a real-time PCR detection system (Universal SYBR green supermix Biorad). All reactions were performed in triplicate to ensure robustness.

The expression levels of the target genes were normalized to the endogenous housekeeping gene β-actin (ACTB) used as an internal control. Relative gene expression was calculated using the 2^−ΔΔCt method.

Table 2: List of primers used:

S.No.	Gene	Sequence (5'-3'')	
		Forward	Reverse
1	β-Actin	GCAGGAGTACGATGAGTCCG	ACGCAGCTCAGTAACAGTCC
2	CD36	TCAATGGCTGTCAGGCGTC	TTGGCTTCAGGGAGACTGTTG
3	PLIN2	GGTGAGTGGCCTGTGTTAGTC	CACACGCCTTGAGAGAAACAG
4	FASN	TTGGAGGGTGTGCCATTCTG	GCTATTCTCTACCGCTGGGG
5	PPAR-γ	GAGGGACGCGGAAGAAGAG	CACAGGCTCCTGTCAGAGTG
6	AdipoQ	TGACGACACCAAAAGGGCTC	ACCTGCACAAGTTCCCTTGG
7	CEBP-α	CCCTTGCTTTTTGCACCTCC	GCTTTCTGGTTCTGACTGGGG
8	RBP4	AATGGTTACTGTCAAAGCAG	AATAGAGATGAAGACCGGATG
9	FATP1	GCCAGGGATCTCTCTCTCCA	GTGCTGGAGCTTGCCTGAT
10	TNF-α	CTATGTCTCAGCCTCTTCTC	CATTTGGGAACTTCTCATCC

Results and discussion

Gene expression changes after 24 hrs of adipocyte treatment was studied to determine drug-specific modulation of genes involved in adipogenesis, lipid metabolism and inflammation. Treatment with pioglitazone (Pio) markedly increased adiponectin (AdipoQ) expression (Fig 1A), indicating enhanced insulin sensitivity. The other two adipokines which are responsible for insulin resistance, RBP-4 and TNF-α did not show statistically significant changes (Fig 1B & C). Markers associated with lipid transport CD36 and FATP1 (Fig 1 C & E) and storage PLIN2 (Fig 1D) did not change upon treatment with Pio. Treatment also reduced PPARγ (Fig 1G), and fatty acid synthase FASN (Fig 1H) without much change in CEBP-α expression (Fig 1I). The reduction in PPARγ, although paradoxical, has been reported earlier (Szychowski et al., 2021).

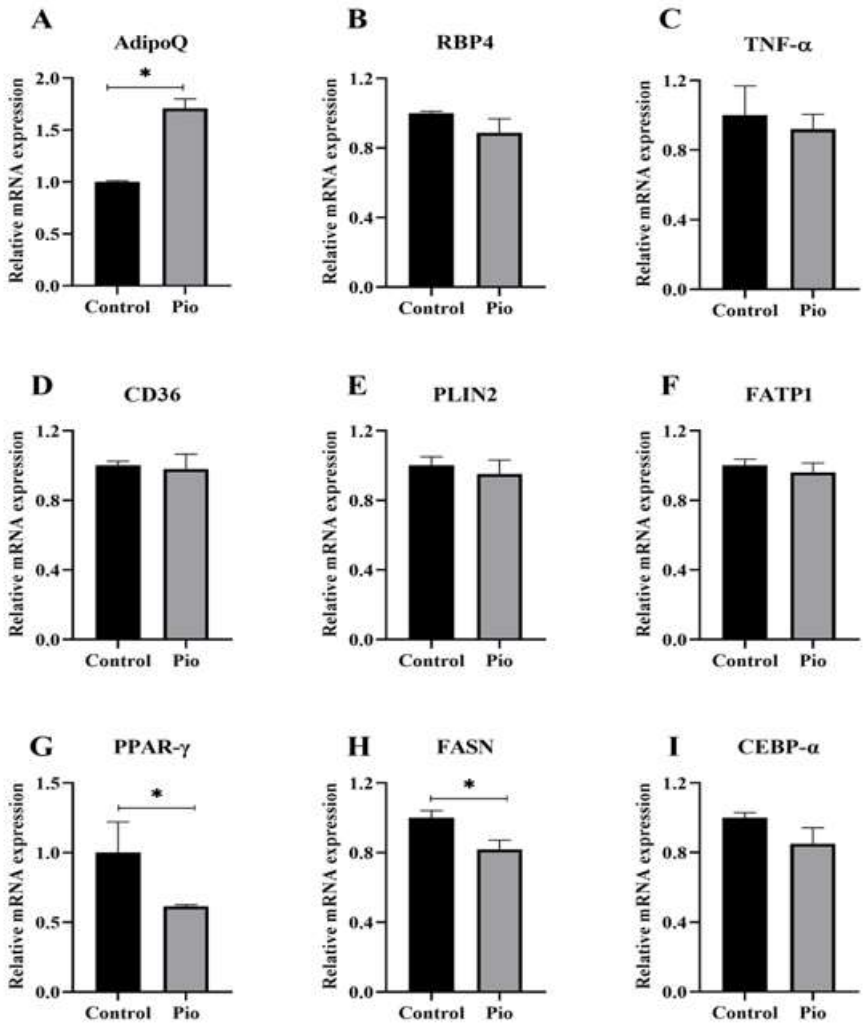

Figure 1: Effect of Pioglitazone (Pio) on adipocytes checked using qRT-PCR

Metformin produced relatively modest changes in gene expression. AdipoQ expression was marginally decreased (Fig 2A), and no change was observed in RBP4 and TNF-α levels (Fig 2B &C). Lipid transport and accumulation markers FATP1 and PLIN2 are increased without much change in CD36 (Fig 2D, E & F). This points to the possibility of metformin increasing lipid accumulation in adipocytes. PPARγ showed pronounced downregulation

(Fig 2G). This is not surprising as metformin is known to have concentration dependent effect on PPARγ in adipocytes (Chen et al., 2018). FASN and CEBP-α expression did not change upon treatment with metformin.

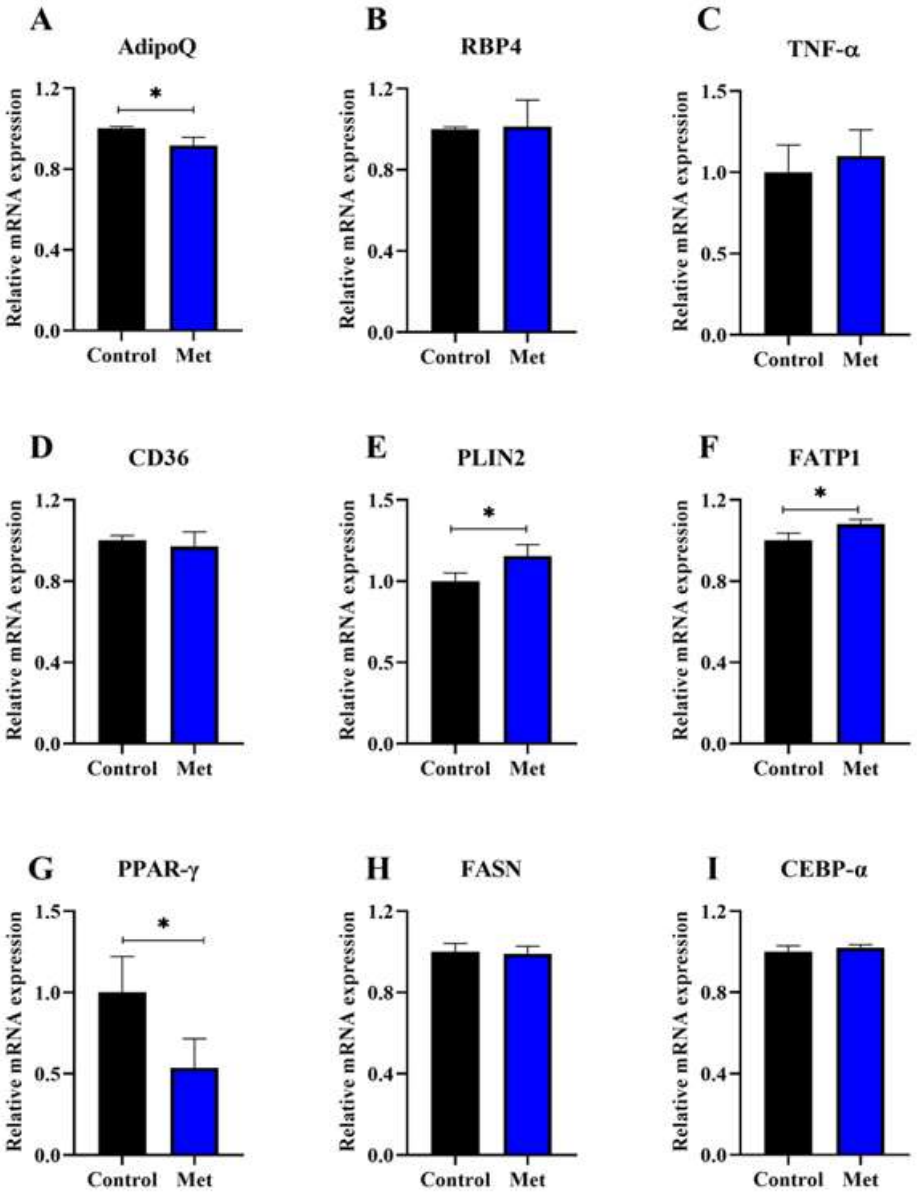

Figure 2: Effect of Metformin (Met) on adipocytes checked using qRT-PCR

Dapagliflozin did not change the expression of any of the adipokines studied (Fig 3A, B & C) However, it did enhance the expression of genes involved in transport and storage of lipids (Fig 3D, E & F). Dapagliflozin increased the expression of CEBP-α while appearing to reduce that of PPARγ without changing FASN (Fig 3G, H & I). Increase in lipid transport and storage markers coupled with enhanced CEBP-α points towards improved insulin sensitivity and lipid accumulation.

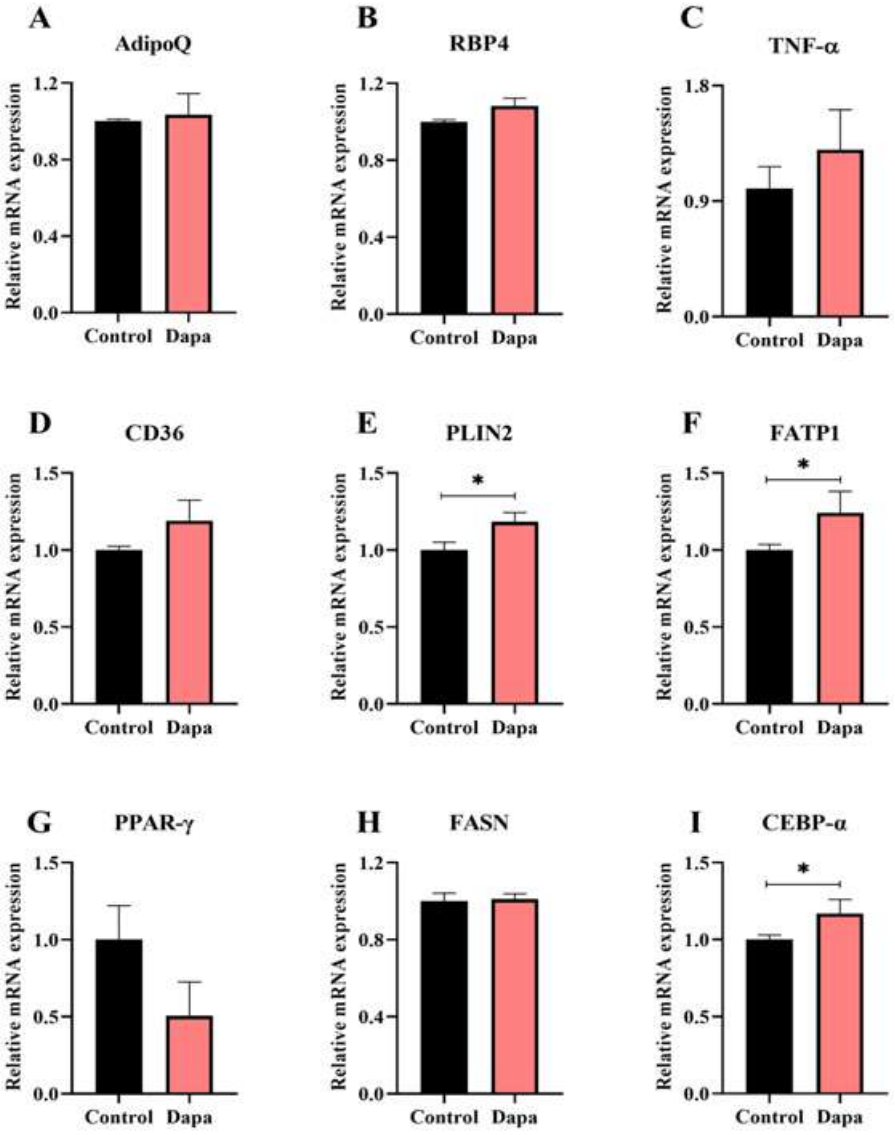

Figure 3: Effect of Dapagliflozin (Dapa) on adipocytes checked using qRT-PCR

Sitagliptin (Sita) significantly increased the expression of RBP4 while marginally reducing AdipoQ and did not alter TNF-α expression (Fig 4A, B & C). This pattern points towards insulin resistance. However, all the lipid transport and storage genes expression was significantly increased (Fig 4D, E & F). In line with this finding we also observed increased PPARγ, FASN and CEBP-α (Fig 4G, H & I). Although PPARγ did not reach statistical significance, the trend could be seen. Taken together, sita produces very interesting changes which is complex and cannot be looked through the binary of increasing or decreasing insulin resistance or sensitivity.

Glimepiride (GLIM) only increased the expression of AdipoQ among the adipokines we looked at (Fig 5A, B & C). Among the storage and transport markers it did appear to increase all but only FATP1 could reach statistical significance (Fig 5D, E & F). Among the metabolic markers it increased CEBP-α only and not PPARγ or FASN.

Overall, the findings demonstrate that antidiabetic compounds differentially regulate adipocyte gene expression. Sitagliptin produced the strongest activation of adipogenic and lipid metabolic pathways, whereas pioglitazone predominantly enhanced AdipoQ expression. Metformin exerted relatively modest transcriptional effects with selective suppression of PPARγ, while dapagliflozin preferentially increased genes associated with lipid uptake despite reduced PPARγ expression and elevated TNF-α. Glimepiride displayed an intermediate profile characterized by moderate enhancement of adipogenic markers accompanied by reduced inflammatory gene expression. These differential transcriptional responses suggest that each antidiabetic agent influences adipocyte metabolism through distinct molecular mechanisms beyond their glucose-lowering actions.

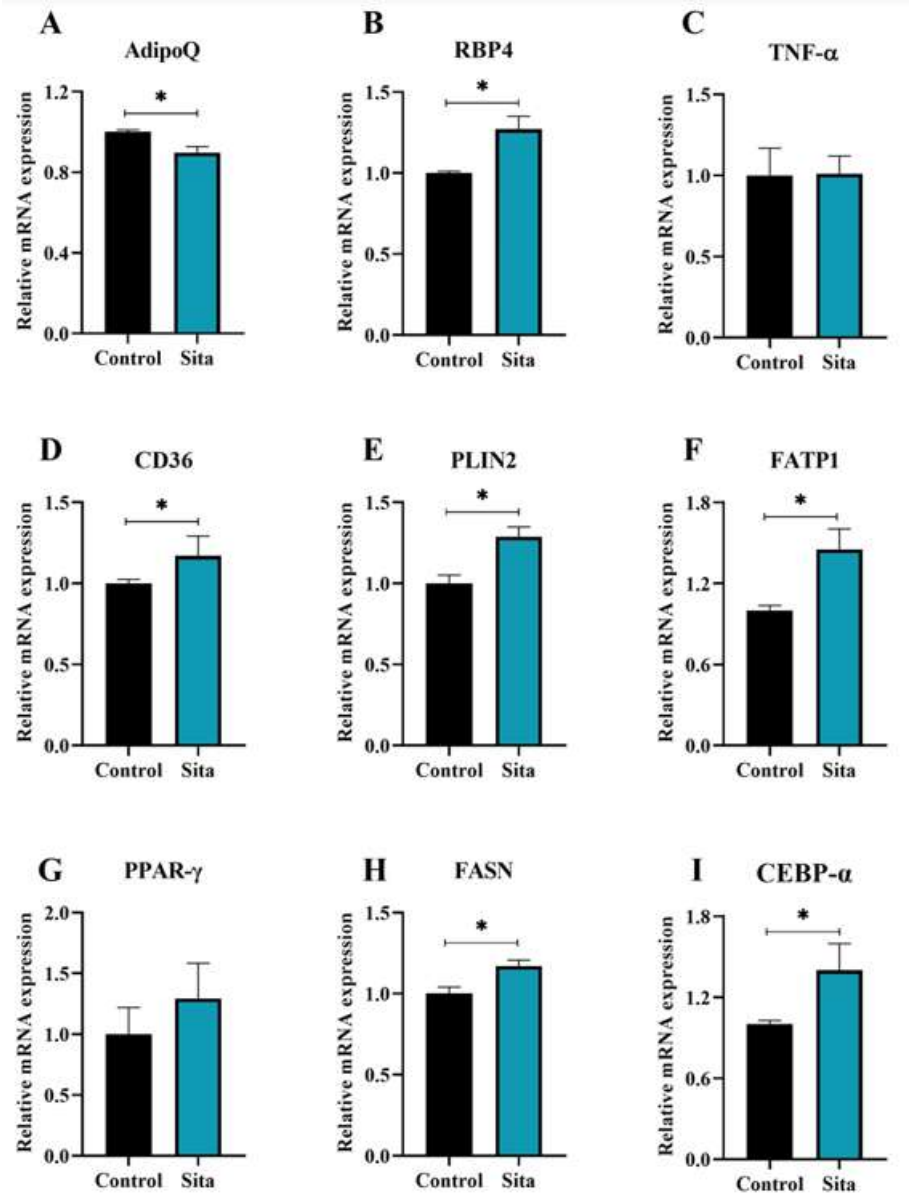

Figure 4: Effect of sitagliptin (Sita) on adipocytes checked using qRT-PCR

Discussion

Adipose tissue is a central regulator of systemic energy homeostasis and insulin sensitivity through its roles in lipid storage, adipokines secretion, and inflammatory signaling. Pharmacological agents used for the treatment of type 2 diabetes may therefore exert metabolic effects beyond glycaemic control by directly modulating adipocyte function. Even though we have several different classes of antidiabetics available for management of diabetes type 2, the number of patients cured or who get drug free once diagnosed with the disease is not large.

Antidiabetic agents differentially regulate the transcriptional landscape of differentiated 3T3-L1 adipocytes, indicating distinct effects on adipogenesis, lipid metabolism, and inflammatory signaling beyond their established glucose-lowering actions. Among the compounds evaluated, sitagliptin induced the most coordinated upregulation of genes associated with adipocyte differentiation and lipid handling, whereas pioglitazone markedly enhanced AdipoQ expression and reduced TNF-α, consistent with improved adipocyte function and an anti-inflammatory phenotype. Metformin exerted comparatively modest transcriptional effects, characterized primarily by suppression of PPARγ expression, while dapagliflozin preferentially increased the expression of genes involved in fatty acid uptake and lipid storage. Glimepiride produced moderate activation of adipogenic and lipid metabolic genes together with reduced inflammatory gene expression.

These findings highlight that antidiabetic drugs possess distinct adipocyte-specific molecular signatures that may contribute to their pleiotropic metabolic effects. The differential regulation of genes involved in adipogenesis, lipid metabolism, and inflammation suggests that the therapeutic benefits of these agents extend beyond glycaemic control and include modulation of adipose tissue function. This also necessitates a more nuanced approach by clinicians in prescribing which class of medicine is better for a patient. Further studies incorporating protein expression analyses, functional metabolic assays, and in vivo validation are warranted to elucidate the molecular mechanisms underlying these transcriptional responses and to determine their physiological relevance in the context of obesity, insulin resistance, and type 2 diabetes mellitus.

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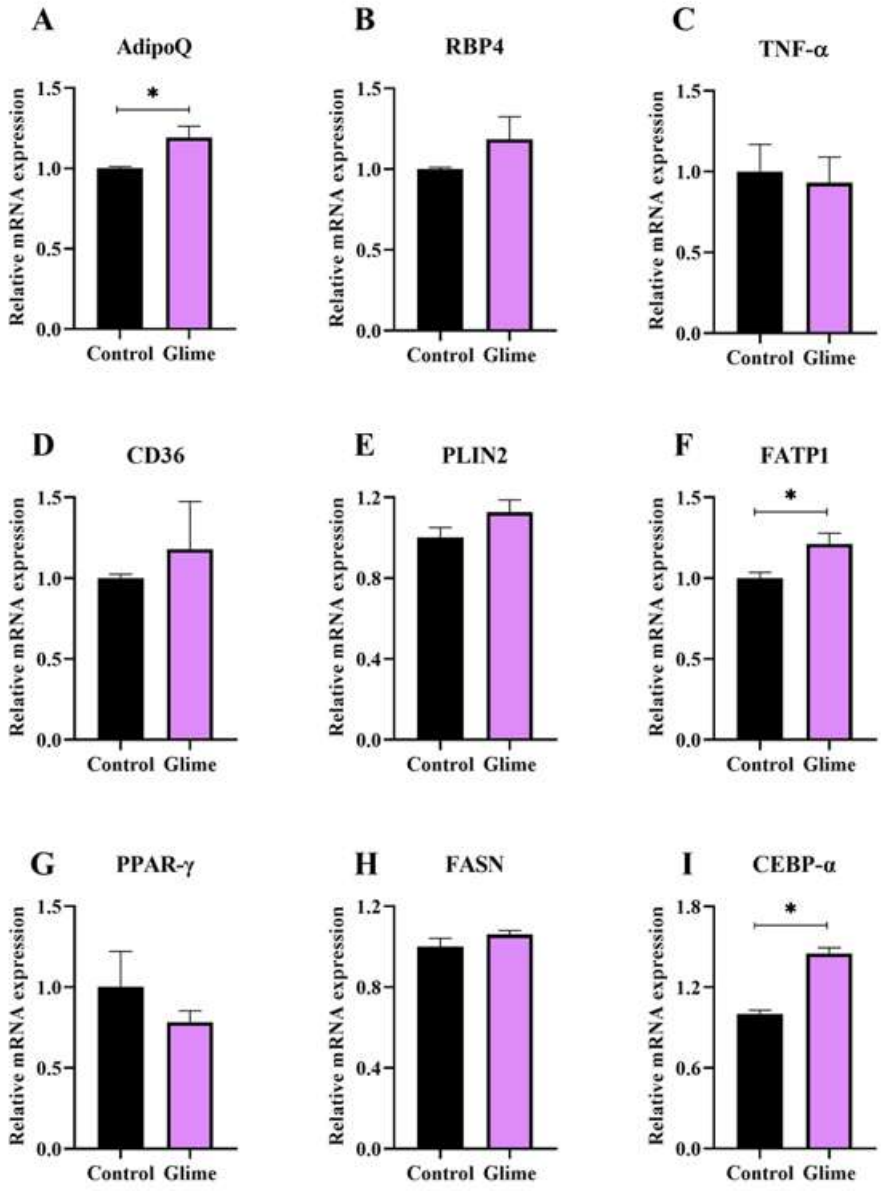

Figure 5: Effect of Glimepiride (Glime) on adipocytes checked using qRT-PCR.

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