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Beyond weight loss: How metabolism in human adipocytes is shaped by GLP-1R agonists and dual GIPR/GLP-1R agonists

Roberto Curto [a,1], Claudia Giglione [b,1], Rossella Cannarella [a], Hygerta Berisha [b], Reham Hattab [b], Rosita A. Condorelli [a], Sandro La Vignera [a], Aldo E. Calogero [a,2,*], Paolo Magni [b,c,2,**]

[a] Department of Clinical and Experimental Medicine, University of Catania, Catania, Italy
[b] Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan 20133, Italy
[c] IRCCS MultiMedica, Sesto San Giovanni, Milan 20099, Italy

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ABSTRACT

Obesity is a chronic disease leading to multiple comorbidities, including type 2 diabetes mellitus (T2DM), cardiovascular disease, and several cancers. Its prevalence has dramatically risen worldwide, making it a major global concern. Although lifestyle interventions remain the cornerstone of management, their effectiveness is often, underscoring the need for novel pharmacological approaches. In this regard, glucagon-like receptor agonists (GLP-1RAs) and the novel dual glucose-dependent insulinotropic polypeptide receptor (GIPR)/GLP-1R agonist, tirzepatide, have shown significant benefits on obesity-related outcomes. Clinical evidence consistently supports their efficacy and safety in promoting weight loss. Furthermore, these agents appear to exert pleiotropic effects by modulating the function of multiple systems, including the cardiovascular, nervous, and gastrointestinal systems. However, the cell type–specific molecular mechanisms underlying their actions in target tissues, particularly adipose tissue, remain incompletely understood. Hence, this review aims to summarize current preclinical evidence on the effects of GLP-1R and dual GIPR/GLP-1R agonists on the function of human-and animal-derived adipocytes. Available data indicate that most studies have been conducted in animal models, both *in vivo* and *in vitro* models, which may not fully recapitulate human adipose tissue pathophysiology. Therefore, future studies should prioritize human-derived cell models to better elucidate and address the pathophysiological and pharmacological downstream effects of GLP-1, GIP, and their agonists in humans.

1. Introduction

Obesity is a chronic and complex disease characterized by excessive and ectopic adipose tissue accumulation that adversely affects human health. It is widely recognized as a major risk factor for several comorbidities, including type 2 diabetes mellitus (T2DM), atherosclerosis, hypertension, obstructive sleep apnea, and metabolic dysfunction-associated steatotic liver disease (MASLD). According to Global Burden of Disease (GBD) estimated, approximately 5 million deaths were attributable to obesity-related comorbidities in 2019 [1]. Currently, obesity affects nearly 800 million people worldwide [2], and its prevalence is projected to reach 1 billion in 2030 [3]. Furthermore, in 2022, 390 million children and adolescents were overweight, of whom 160 million were obese. Childhood obesity is also associated with an increased risk of developing T2DM and cardiovascular disease later in life [4]. Collectively, these data underscore obesity as a global public health emergency, highlighting the urgent need for coordinated strategies to reverse this trend.

Obesity is a multifactorial disease caused by genetic, epigenetic, environmental, and psychosocial determinants. The rising prevalence of

* Correspondence to: Dipartimento di Medicina Clinica e Sperimentale, Università degli Studi di Catania, AOUP "G. Rodolico, San Marco" – ed. 4, 2° p. stanza 17 - via S. Sofia 278, Via S. Sofia 78, Catania, CT 95125, Italy.
** Correspondence to: Dipartimento di Scienze Farmacologiche e Biomolecolari "Rodolfo Paoletti" (DiSFeB), Università degli Studi di Milano, Via G. Balzaretti 9, 20133 Milano (MI), IRCCS MultiMedica, Via Milanese, 300, Sesto San Giovanni, MI 20099, Italy.
E-mail addresses: aldo.calogero@unict.it (A.E. Calogero), paolo.magni@unimi.it (P. Magni).
[1] These authors equally contributed to this work as co-first authors
[2] These authors equally contributed to this work as co-last authors

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obesity is closely linked to sociocultural changes in modern societies. In this context, the WHO has defined obesity as a societal rather than solely an individual responsibility [4]. Therefore, a key priority in addressing obesity in the development of supportive environments that promote healthy lifestyles [5], with particular emphasis on early prevention and management during childhood and adolescence.

The clinical management of obesity has traditionally relied on behavioral interventions aimed at promoting healthy lifestyles, including adherence to the Mediterranean diet. Although, these approaches remain the cornerstone of prevention and treatment, they are often associated with modest and discouraging results [6,7]. Among dietary strategies, the very low-calorie ketogenic diet (VLCKD), also known as very-low-energy ketogenic therapy (VLEKT), has emerged as an effective option in selected patients [8]. Bariatric surgery represents another effective therapeutic approach for severe obesity [9].

Pharmacological strategies for obesity have been pursued since the 20th century, albeit with limited long-term success. Early treatments, including thyroid hormones and dinitrophenol, were discontinued due to the risk of thyrotoxicosis and toxic effects, respectively [10]. Amphetamines were later introduced for their appetite-suppressing effects but became strictly regulated due to their addictive potential and cardiovascular risks [10,11]. The combination of bupropion, an antidepressant, and naltrexone, an opioid receptor antagonist, has been approved for obesity management, although its efficacy in inducing weight loss is modest [10,12]. Additional targets, including leptin, neuropeptide Y, and melanocortin−4 receptor agonists, have been extensively investigated but clinical outcomes have been disappointing [13]. Several centrally acting molecules, such as rimonabant, a cannabinoid receptor antagonist, sibutramine, a serotonin and norepinephrine reuptake inhibitor, and fenfluramine, a serotonergic agent, were also introduced but subsequently withdrawn due to safety concerns [14].

The advent of glucagon-like peptide−1 receptor agonists (GLP−1RAs) represents a milestone in the advancement of obesity management. These molecules mimic endogenous GLP−1 but exhibit prolonged activity, leading to delayed gastric emptying and reduced food intake through central and possibly peripheral mechanisms. Among GLP−1RAs, liraglutide and semaglutide have been approved by both the Food and Drug Administration and the European Medicines Agency for the treatment of obesity and T2DM. In the SCALE trial, conducted in patients with obesity (mean BMI 37 kg/m^2), liraglutide treatment resulted in a mean body weight reduction of 4.7% (1.8 mg/day) and 6% (3 mg/day), along with reductions in waist circumference of 4.8% (1.8 mg/day) and 6.1% (3 mg/day) respectively, compared with placebo (2.0% and 2.7%)after 56 weeks [15]. Similarly, the STEP−5 trial demonstrated that semaglutide (2.4 mg/week) reduced mean body weight by 15.2%, and waist circumference by 14.4 cm after 104 weeks in patients with a mean BMI of 38.5 kg/m^2, compared with reductions of 2.6% and 5.2 cm in the placebo group [16]. These findings confirm the robust efficacy of GLP−1RAs in promoting weight loss.

More recently, tirzepatide (TZP), a dual glucose-dependent insulinotropic polypeptide receptor (GIPR)/GLP−1RA, has been approved for obesity treatment. Clinical trials have demonstrated its substantial efficacy in reducing body weight, and reductions of −15%, −19.5%, and 20.9% at doses of 5, 10, and 15 mg/week, respectively compared with −3.1% with placebo after 72 weeks in patients with mean BMI 38 Kg/m^2 have been reported [17]. Greater weight loss has also been observed when TZP was compared with insulin glargine-based regimens combined with other anti-diabetic drugs (mean body weight difference: 5 mg/week TZP: −9 kg; 10 mg/week TZP: −11.4 kg; 15 mg/week TZP: −13.5 kg; vs. insulin plus other antidiabetic drugs: +1.9 kg) in patients with inadequately controlled T2DM by any combination of oral glucose-lowering medications (metformin, sulfonylurea, or sodium-glucose co-transporter−2 inhibitor) and BMI 25 Kg/m^2 (mean BMI 32.6Kg/m^2) after 52 weeks [18], and 1 mg/week semaglutide (mean body weight difference: 5 mg/week TZP: −7.8 kg; 10 mg/week TZP: −10.3 kg; 15 mg/week TZP: −12.4 kg; *vs* 1 mg/week semaglutide: −6.2 kg) in patients with inadequately controlled T2DM by metformin (1500 mg/day) and BMI 25 Kg/m^2 (mean BMI 34.2 Kg/m^2) after 40 weeks [19]. Mean waist circumference also significantly decreased in patients treated with TZP (5 mg/week: −14 cm; 10 mg/week: −17.7 cm; 15 mg/week: −18.5 cm) vs. placebo group (−4 cm) after 72 weeks [17], and was more effective compared to 1 mg/week semaglutide (5 mg/week TZP: −6.9 cm; 10 mg/week TZP: −9.6 cm; 15 mg/week TZP: −9.9 cm; vs 1 mg/week semaglutide: −5.6 cm) after 40 weeks [19]. The results of the aforementioned clinical studies are summarized in Table 1.

Moreover, accumulating evidence indicates that GLP−1RAs and dual GIPR/GLP−1RA exert beneficial effects on multiple obesity-related comorbidities. Recent meta-analyses have shown that these therapies are associated with a reduced risk of cardiovascular [20,21], respiratory [22], hepatic [23], and renal [24] diseases.

In addition, GLP−1 and GIP receptors are widely expressed across various human tissues [25], suggesting that these molecules may exert direct, pleiotropic effects beyond their metabolic actions. Consistent with this hypothesis, recent studies have reported that GLP−1RAs and dual GIPR/GLP−1RA may exert direct cardioprotective effects independent of weight loss, as shown in human-derived immortalized AC16 cardiomyocytes and ventricular cardiomyocyte explants, respectively [26,27].

Adipose tissue is now widely recognized as an active endocrine organ that secretes a variety of bioactive molecules, including adipokines, inflammatory mediators, and metabolites that influence metabolic homeostasis [28].

However, whether GLP−1RA and dual GIPR/GLP−1RAs directly modulate human adipocyte function remains incompletely understood. This review aims to examine the molecular signaling pathways regulated by GLP−1RAs and dual GIPR/GLP−1RA in adipose tissue, with a particular focus on adipocytes, its principal cellular component.

To address this aim, the following key terms were used to conduct literature searching: “GLP−1”, “GIP”, “GLP−1 receptor agonist”, “dual GIPR/GLP−1R agonist”, “exenatide”, “exendin−4”, “liraglutide”, “dulaglutide”, “semaglutide”, or “tirzepatide” in conjunction with “preadipocyte”, “preadipocyte differentiation”, “adipocyte”, “human adipocytes”, “lipid metabolism”, “adipokines”, “oxidative stress”, “redox homeostasis”, “inflammation”, insulin resistance”, “glucose uptake”, “fat browning”, “thermogenesis”, “3T3-L1 cells”. Only original research articles published in English were considered. Studies were selected based on their relevance to adipocyte differentiation, lipid metabolism, adipokine regulation, redox balance, inflammatory processes, insulin resistance, fat browning, and thermogenesis in adipocytes or adipose tissue, focusing on animal-derived *in vitro* and *in vivo* models, or human-derived experimental *in vitro* models. The obtained experimental evidence has been discussed within the frame of related clinical trials following a reverse translation approach. The last search was conducted on 10 December 2025.

In the next sections, we examine the effects of GLP−1, GIP, and their analogs on key adipocyte processes, including differentiation, proliferation, lipogenesis, lipolysis, thermogenesis, and the secretion of adipokines and inflammatory mediators.

2. Molecular mechanisms associated with GIP and GLP−1 systems

GLP−1 is an incretin secreted by enteroendocrine L-cells located in the distal small intestine within minutes after nutrient ingestion. In these cells, proglucagon is processed by prohormone convertases to generate the bioactive forms of GLP−1, namely GLP−1(7−37) and GLP−1(7−36) amide. Both peptides contain an alanine residue at position 2, rendering them substrates for dipeptidyl peptidase−4 (DPP−4), which rapidly degrades them into the inactive GLP−1(9−37) and GLP−1(9−36)amide within minutes [29].

Among its principal physiological actions, GLP−1 enhances glucose-

Table 1
Summary of clinical evidence of glucagon-like peptide−1receptor agonists (GLP−1RAs) and tirzepatide (TZP) on body weight and waist circumference reductions.

Clinical trial	Study population	Treatment	Results	Reference
• SCALE	Patients with obesity (mean BMI 37 Kg/m^2)	1.8 mg/day or 3 mg/day liraglutide vs. placebo for 56 weeks	• *1.8 mg/day liraglutide group:* mean ↓ 4.7% BW and mean ↓ 4.8% WC • *3 mg/day liraglutide group:* mean ↓ 6% BW and mean ↓ 6.1% WC • *Placebo group:* mean ↓ 2% BW and mean ↓ 2.7% WC	[15]
• STEP−5	Patients with obesity (mean BMI 38.5 Kg/m^2)	2.4 mg/week semaglutide vs. placebo for 104 weeks	• *2.4 mg/week semaglutide group:* mean ↓ 15.2% BW and mean ↓ 14.4 cm WC • *Placebo group:* mean ↓ 2.6% BW and mean ↓ 5.2 cm WC	[16]
• SURMOUNT−1	Patients with mean BMI ≥ 38 Kg/m^2	5 mg/week, or 10 mg/week, or 15 mg/week TZP vs. placebo for 72 weeks	• *5 mg/week TZP group:* mean ↓ 15% BW and mean ↓ 14 cm WC • *10 mg/week TZP group:* mean ↓ 19.5% BW and mean ↓ 17.7 cm WC • *15 mg/week TZP group:* mean ↓ 20.9% BW and mean ↓ 18.5 cm WC • *Placebo group:* mean ↓ 3.1% BW and mean ↓ 4 cm WC	[17]
• SURPASS−4	Patients with BMI ≥ 25 Kg/m^2 (mean BMI 32.6 Kg/m^2) and inadequately controlled T2DM by any combination of oral glucose-lowering medications (metformin, sulfonylurea, or sodium-glucose co-transporter−2 inhibitor)	5 mg/week, or 10 mg/week, or 15 mg/week TZP vs. insulin glargine + other anti-diabetic drugs for 52 weeks	• *5 mg/week TZP group:* mean ↓ 9 Kg BW • *10 mg/week TZP group:* mean ↓ 11.4 Kg BW • *15 mg/week TZP group:* mean ↓ 13.5 Kg BW • *Insulin glargine + other anti-diabetic drugs group:* mean ↑ 1.9 Kg BW	[18]
• SURPASS−2	Patients with BMI ≥ 25 Kg/m^2 (mean BMI 34.2 Kg/m^2) and inadequately controlled T2DM by metformin	5 mg/week, or 10 mg/week, or 15 mg/week TZP vs. 1 mg/week semaglutide for 40 weeks	• *5 mg/week TZP group:* mean ↓ 7.8 Kg BW and mean ↓ 6.9 cm WC • *10 mg/week TZP group:* mean ↓ 10.3 Kg BW and mean ↓ 9.6 cm WC • *15 mg/week TZP group:* mean ↓ 12.4 Kg BW and mean ↓ 9.9 cm WC • *1 mg/week semaglutide group:* mean ↓ 6.2 Kg BW and mean ↓ 5.6 cm WC	[19]

Abbreviations: BMI, body mass index; BW, body weight; TZP, tirzepatide; T2DM, type 2 diabetes mellitus; WC, waist circumference.

dependent insulin secretion from pancreatic β-cells, suppresses glucagon secretion, inhibits ß-cell apoptosis, slow gastric emptying, and reduces food intake.

GLP−1 exerts its effects through its specific receptor which belongs to class B G protein-coupled receptors (GPCRs) [30]. GLP−1Rs are expressed in multiple tissues, including the pancreas, heart, kidney, lung, skeletal muscle, and central nervous system [25], supporting the pleiotropic nature of GLP−1 signaling. Upon activation, GLP−1R primarily couples to $G\alpha_s$, leading to stimulation of adenylate cyclase (AC) and subsequent production of cyclic adenosine monophosphate (cAMP). Elevated cAMP levels activate downstream signaling pathways, including cAMP-dependent protein kinase (PKA) and exchange protein activated by cAMP (EPAC), which play key roles in the regulation of glucose-dependent insulin secretion by pancreatic β-cells [31] (Fig. 1, **blue arrows**).

In addition, GLP−1 can enhance insulin secretion through activation of the $G\alpha_q$/phospholipase-C (PLC)/protein kinase C (PKC) pathway, thereby modulating intracellular Ca^{2+} influx in β-cells [32] (Fig. 1, **yellow arrows**).

Furthermore, GLP−1R activation can stimulate the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) signaling pathway via Gβγ subunits, promoting β-cells survival and improving insulin sensitivity in peripheral tissues [31] (Fig. 1, **grey arrows**). Anti-apoptotic effects in β-cells may also be mediated by GLP−1R interaction with the proto-oncogene tyrosine-protein kinase Src, leading to transactivation of the epidermal growth factor receptor (EGFR) and subsequent activation of the PI3K/AKT pathway [33] (Fig. 1, **grey arrows**).

Upon prolonged GLP−1R activation, ß-arrestin−1 and −2 are recruited, promoting receptor internalization and subsequent lysosomal degradation, attenuating receptor signaling, or facilitating receptor recycling to the plasma membrane [32]. In addition to these regulatory processes, β-arrestin-independent mechanism of GLP−1R internalization has also been described [34–36] (Fig. 1, **green arrows**).

Notably, Quoyer et al. demonstrated that GLP−1-induced activation of extracellular signal-regulated kinases (ERK) –1/2 in murine pancreatic β-cells involves ß-arrestin−1. Specifically, the early phase of ERK1/2 activation is dependent on PKA signaling (Fig. 1, blue arrows), while ß-arrestin−1 mediates sustained ERK1/2 activation during the late phase, contributing to anti-apoptotic effects [37] (Fig. 1, **purple arrows**).

Recent advances in structural pharmacology have provided a mechanistic framework to explain the functional diversity of GLP−1R agonists. Biased agonism at the GLP−1 receptor arises from ligand-specific stabilization of distinct receptor conformations, which differentially regulate downstream signaling pathways. Particularly, the coordinated interplay between the extracellular domain (ECD) and the transmembrane (TM) core governs the balance between G protein coupling and β-arrestin recruitment [38,39].

Structural studies indicate that variations in how the ligand N-

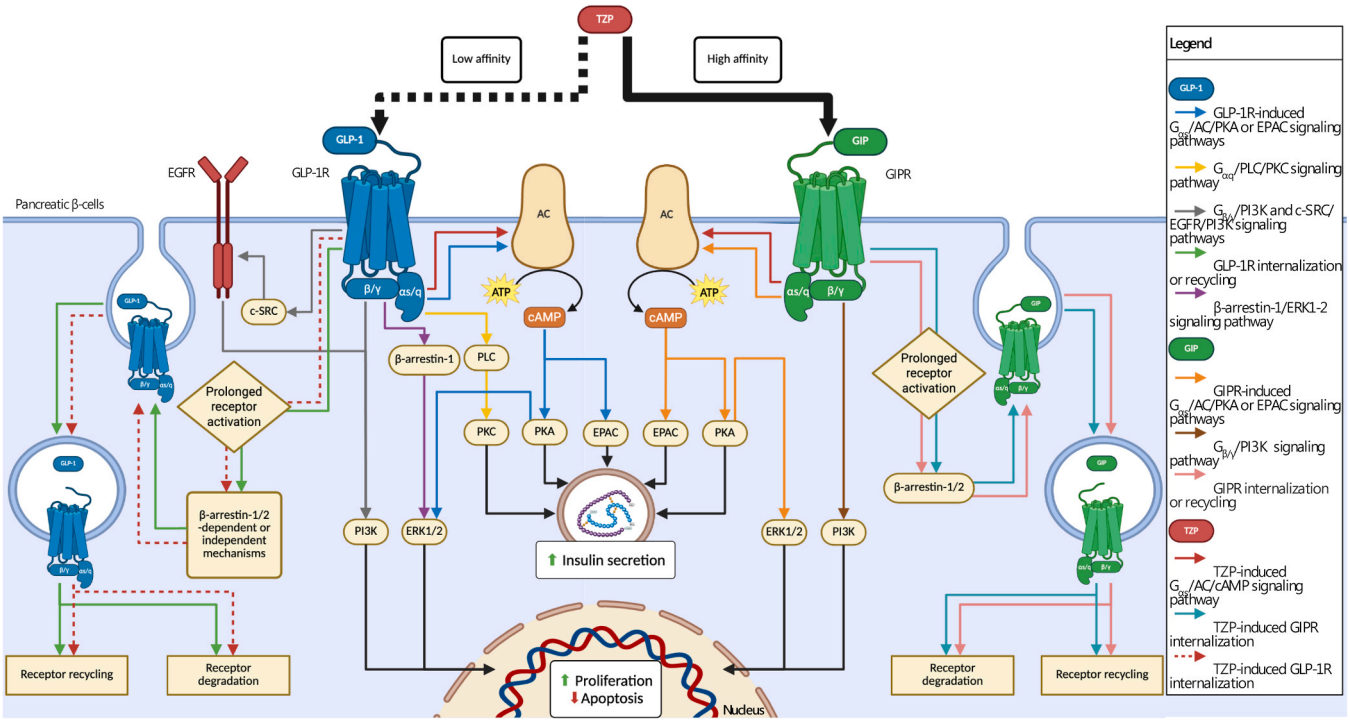

Fig. 1. GLP−1, GIP, and tirzepatide signaling pathways in pancreatic β-cells. GLP−1 binds GPL−1R that couples $G_{\alpha s}$ leading to cAMP production by activating AC. Consequently, cAMP induces downstream signaling including PKA and EPAC, regulating insulin secretion (blue arrows). PKA also stimulates ERK1/2 promoting β-cell anti-apoptotic effect (blue arrows). ERK1/2 can be activated by β-arrestin−1 (purple arrows). $G_{\alpha q}$ subunit induces PLC/PKC signaling pathway which is involved in insulin secretion (yellow arrows). β-cells proliferation and survival processes are regulated by PI3K that can be induced directly by GLP−1R, by coupling $G_{\beta/\gamma}$, or indirectly via c-Src/EGFR stimulation (grey arrows). Prolonged GLP−1R activation promotes receptor recycling or degradation via β-arrestin−1/2-dependent and -independent mechanisms (grey arrows). Similarly, GIP can induce $G_{\alpha s}$/AC/cAMP/PKA or EPAC (orange arrows) and $G_{\beta/\gamma}$/PI3K (brown arrows) that are respectively involved in insulin secretion and β-cell apoptosis regulation. By contrast, GIPR internalization or recycling is only mediated by β-arrestin−1/2 (pink arrows). TZP stimulates cAMP production by interacting with both GLP−1R and GIPR (red arrows). TZP elicits lower β-arrestin-induced GLP−1R internalization than GLP−1 (red dotted arrows), and a comparable GIPR degradation compared to GIP (cyan arrows). ***Abbreviations*:** AC, adenylate cyclase; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; c-SRC, proto-oncogene tyrosine-protein kinase Src; EGFR, epidermal growth factor receptor; EPAC, exchange protein activated by cAMP; ERK1/2, extracellular signal-regulated kinase 1/2; GIP, glucose-dependent insulinotropic polypeptide; GIPR, GIP receptor; GLP−1, glucagon-like peptide; GLP−1R, GLP−1 receptor; GLP−1RA, GLP−1R agonist; PI3K, phosphoinositide 3-kinase; PKA, cAMP-dependent protein kinase; PKC protein kinase C; PLC, phospholipase C; TZP, tirzepatide.

terminus engages the TM binding pocket, together with specific interactions with the ECD, influence receptor activation kinetics as well as post-activation trafficking, including receptor internalization and recycling [40,41].

These conformational dynamics ultimately shape both the intensity and duration of signaling [42]. Different GLP−1R agonists exploit these structural determinants to generate distinct signaling profiles. For example, exendin−4 displays altered N-terminal interactions within the receptor core that stabilize signaling-competent conformations while modulating internalization kinetics [43,44]. In contrast, the clinical efficacy of semaglutide appears to be primarily driven by enhanced plasma stability and prolonged receptor residence time, rather than by a marked intrinsic signaling bias [44,45].

Overall, GLP−1R biased agonism reflects ligand-dependent conformational selection. Elucidating these structural mechanisms is essential to interpret the differences in efficacy, durability, and tolerability of currently available incretin-based therapies.

Conversely, GIP is a 42-amino acid incretin secreted by enteroendocrine K-cells located in the proximal small intestine. Similar to GLP−1, GIP contains an alanine residue at position 2, making it a substrate for DPP−4-mediated degradation.

GIP exerts its biological effects through the GPCR, a G protein–coupled receptor expressed in pancreatic islet β-cells, adipose tissue, heart, and brain [25]. Upon activation, GIPR engages multiple intracellular signaling pathways, including the cAMP/PKA and EPAC cascades, as well as ERK1/2 (Fig. 1, **orange arrows**), and PI3K/AKT pathways (Fig. 1, **brown arrows**), thereby promoting insulin secretion, ß-cells survival, and proliferation [33].

In contrast to GLP−1R, GIPR signaling appears to be predominantly regulated through β-arrestin–mediated receptor internalization, which contributes to signal attenuation [33] (Fig. 1, **pink arrows**).

It has been demonstrated that mice lacking GIPR (*Gipr-/-*) and fed with high-fat diet (HFD) are protected against the development of obesity and insulin resistance. Furthermore, *Gipr*$^{-/-}$ mice exhibit increased thermogenesis and improved glucose homeostasis [46]. Consistent findings have also been reported using an antagonist of GIPR [47].

Additional evidence indicates that GIP can enhance blood flow and nutrient uptake, promote lipid deposition in lean individuals, and increase triglyceride content in adipose tissue [48–50]. Collectively, these findings suggest that GIP plays a role in the regulation of adipocyte metabolism, energy expenditure, and insulin sensitivity.

Current evidence suggests that GIP may potentiate the metabolic effects mediated by GLP−1. In this regard, preclinical and early clinical studies have demonstrated that unimolecular co-agonist targeting both GIP and GLP−1 receptors may provide greater efficacy in the management of obesity and its associated comorbidities compared with GLP−1RA alone [51].

For dual GIPR/GLP−1RAs, numerous clinical trials have evaluated the efficacy and safety of TZP. However, the molecular mechanisms underlying its pharmacological effects remain incompletely understood. Early clinical trials investigating the co-infusion of GIP and GLP−1 in

patients with T2DM or overweight did not demonstrate any additive effects on energy expenditure, appetite, and insulin secretion compared with GLP−1 alone [52–55]. These findings suggest that TZP does not act as a simple dual agonist of GIPR and GLP−1R, but rather though more complex mechanisms.

Recent evidence indicates that TZP exhibits biased affinity for the two receptors. In HEK293 cells, TZP shows approximately five-fold lower affinity for GLP−1R compared with native GLP−1(7−36), because TZP engages GLP−1R through a non-native binding mode that preferentially stabilizes G protein–coupled conformations [56,57], while displaying a similar affinity for GIPR compared with GIP (1−42). Despite this, TZP effectively stimulates cAMP production through both receptors (Fig. 1**, red arrows**), although it induces less GLP−1R internalization than GLP−1(7–36) (Fig. 1**, red dotted arrows**). Conversely, TZP promotes β-arrestin recruitment at GIPR to an extent comparable to GIP(1–42) (Fig. 1**, cyan arrows**) [58].

These observations suggest that the prolonged stimulation of both receptors provided by TZP may result in sustained GLP−1R signaling exceeding that of native GLP−1, while functionally modulating GIPR activity, potentially toward antagonism via β-arrestin–mediated mechanisms [58]. In line with this hypothesis, a clinical trial combining a GIPR antagonist with a GLP−1RA showed improvements in metabolic parameters and body weight loss in obese patients [59].

Nevertheless, further studies employing physiologically relevant human-derived cell models are required to more accurately elucidate the mechanisms of action of TZP across target human tissues.

The following sections will focus on the effects of GIP- and GLP−1 on adipocyte differentiation, metabolic regulation, and the molecular machineries controlling oxidative stress response, inflammation, adipokine secretion, adipose tissue browning, and thermogenesis, drawing on evidence from both animal models and human-derived adipocytes.

2.1. Adipocyte differentiation

2.1.1. Brief introduction to adipocyte differentiation

Adipocytes arise through a tightly regulated and multistep differentiation process in which preadipocytes progressively mature into fully differentiated adipocytes via temporally coordinated ad sequential transcriptomic changes triggered by diverse stimuli. Several factors, including age, sex, and lifestyle, have been shown to modulate this process either positively or negatively [60].

Induction of adipocyte differentiation activates specific transcription factors that orchestrate chromatin remodeling, ultimately establishing adipocyte-specific gene expression profiles and phenotypes [61]. Notably, the differentiation trajectory varies according to the cell lineage of origin [62] and the commitment of preadipocyte to distinct adipocyte subtypes, namely white [63], brown [64], or beige [65] adipocytes, which represent the principal adipocyte phenotypes known in humans.

Historically, the peroxisome proliferator-activated receptor (PPAR)-γ has been recognized as the master regulator of adipocyte differentiation. It functions in concert with key transcriptional factors, including members of the CAAT enhancer binding protein (C/EBP) family (α, β, and δ), adipocyte differentiation and determination factor 1 (ADD−1), and high-mobility group protein I-C (HMGI-C), all of which are critically involved in adipogenesis [66].

In the early phase, cAMP response element-binding protein (CREB), together with C/EBP-β and C/EBP-δ, initiates the first wave of transcriptional changes leading to activation of downstream adipogenic regulators [67]. In contrast, during the late stage, C/EBP-α cooperates with PPAR-γ to drive terminal differentiation, coupled with the induction of key metabolic genes [68].

As differentiation progresses, preadipocyte markers such as DPP−4 and cluster of differentiation 9 (CD9) are progressively downregulated [69], whereas adipocyte-specific markers - including fatty acid-binding protein 4 (FABP4), glucose transporter 4 (GLUT4), and perilipin−1 (PLIN1) - are upregulated in mature adipocytes [70]. These proteins are abundantly expressed in differentiated adipocytes, where they regulate lipid transport and homeostasis, as well as glucose uptake.

More recent evidence has identified additional regulators of adipogenesis, including insulin-like growth factor−1 (IGF−1), macrophage colony stimulating factor (M-CSF), prostaglandins, glucocorticoids, and fatty acids. In contrast, inhibitory signals include transforming growth factor (TGF)-β, pro-inflammatory cytokines, and growth hormone (GH) [71].

Taken together, these cues drive a coordinated transcriptional reprogramming that underlies the structural and functional transition of preadipocytes into a mature phenotype.

2.1.2. Evidence on modulation of adipocyte differentiation by GIP, GLP−1, and their agonists

Hormone responsiveness represents a peculiar feature of adipocytes. Several endocrine signals, including insulin and IGF−1, thyroid hormones, glucocorticoids, GH and sex steroids, play critical roles in adipocyte differentiation and commitment, regulation of fat distribution, adipose tissue remodeling, and modulation of metabolic functions [72]. In particular, white adipocytes act as metabolic sensors, integrating hormonal cues such as the incretins GIP and GLP−1 to regulate energy balance [73].

In a pioneering study, Eckel et al. demonstrated that murine 3T3-L1 preadipocytes incubated with GIP (10^{-11}, 10^{-10}, and 10^{-9} M) for 2 h exhibited increased expression and activity of lipoprotein lipase (Lpl), a key enzyme involved in free fatty acid (FFA) uptake and predominately expressed in mature adipocytes [74]. These findings provided early evidence supporting a role for GIP in adipocyte biology and prompted subsequent investigations into the expression of the GIPR and its downstream signaling pathways during adipocyte differentiation.

Consistently, Yip et al. [75] reported higher Gipr mRNA and protein expression in isolated rat adipocytes and differentiated 3T3-L1 adipocytes compared with undifferentiated cells. Functional Gipr activity during adipocyte differentiation was further supported by increased intracellular cAMP accumulation following exposure to 10^{-8} M GIP for 15 min, indicating active GIPR signaling [75].

Similarly, Song et al. [76] detected Gipr expression in both differentiated murine 3T3-L1 cells and primary human adipocytes. Notably, Gipr expression was low in undifferentiated 3T3-L1 cells and progressively increased during adipocyte differentiation, whereas Ppar-γ expression was induced at later stages. Exposure to 2×10^{-9} M GIP during 3T3-L1 differentiation enhanced Fabp4 expression compared with insulin stimulation alone (1 μg/mL) supporting a pro-adipogenic role of GIP. At the signal level, GIP induced Akt activation in a dose-dependent manner (2×10^{-10}, 2×10^{-9}, 2×10^{-8}, and 2×10^{-7} M, 10-minutes exposure), in both differentiated 3T3-L1 and human adipocytes. Moreover, prolonged GIP exposure (2×10^{-8} M for 4 days), in the presence of adipogenic stimuli, promoted GLUT4 translocation to the plasma membrane in differentiated 3T3-L1 cells. This effect is consistent with activation of the Akt pathway and enhanced insulin-responsive glucose uptake [76].

By contrast, Kim et al. [77] reported that both Gipr transcript and protein levels increased predominantly during the late phase of 3T3-L1 adipocyte differentiation, induced by an adipogenic cocktail in the presence or absence of GP (10^{-8} and 10^{-7} M) over 7 days. Mechanistically, Gipr upregulation was shown to depend on transcriptional control mediated by Ppar-γ and epigenetic remodeling, specifically histone H3/H4 acetylation. Indeed, Ppar-γ, together with acetylated histones H3/H4, was found to bind the Gipr promoter, thereby enhancing its transcription in differentiated 3T3-L1 adipocytes [77].

Taken together, these findings support a relevant role for GIP and its receptor in adipogenesis and adipocyte metabolism, through mechanisms that partially overlap with insulin signaling pathways.

Interestingly, in a human-derived adipocyte cell line, Simpson-Golabi-Behmel syndrome (SGBS) preadipocytes, GIPR mRNA

expression increased during the early phase of differentiation and was followed by upregulation of *FABP4*. In contrast, during later stages *GIPR* expression declined relative to *FABP4* levels. Functionally, intracellular cAMP accumulation in response to GIP stimulation (10^{-11} – 10^{-6} M in the presence of 10^{-10} M 3-isobutyl−1-methyl-xanthine for 12 min) was higher in differentiated compared with undifferentiated SGBS cells, suggesting enhanced GIPR functional activity upon differentiation [78].

Another relevant regulator of adipocyte differentiation is GLP−1, which has been proposed to exert adipogenic effects. Several studies using the murine 3T3-L1 preadipocyte cell line have investigated the role of GLP−1 in adipogenesis and adipocyte metabolism. In this context, GLP−1 and its long-acting analog, liraglutide (10^{-9}–10^{-7} nM) were shown to promote adipogenesis by enhancing preadipocyte proliferation and reducing apoptosis through GLP−1R–dependent activation of Erk1/2, protein kinase C (PkC)-β II, and Akt signaling in pathways.

Notably, *Glp−1r* expression increased during the late phase of adipocyte differentiation, in parallel with Ppar-γ expression. Consistent with this *in vitro* findings, HFD-fed mice treated with liraglutide (100 μg/kg, twice daily for the final 17 days) exhibited increased adipogenic capacity, indicative of enhanced preadipocyte differentiation.

Collectively, these data suggest that pharmacological activation of GLP−1R may promote adipose tissue expansion predominantly through hyperplastic mechanisms [79].

GLP−1-mediated differentiation of murine 3T3L−1 preadipocytes has also been linked to activation of the Wnt/β-catenin signaling pathway, a key regulator of adipocyte differentiation. Specifically, incubation with GLP−1 (10^{-8} and 5 $\times 10^{-8}$ M) for 3 h to 4 days induced Wnt4 expression, promoting sequestration of β-catenin at the plasma membrane and thereby limiting its nuclear translocation. This shift facilitated the expression of adipogenic transcription factors, ultimately promoting 3T3-L1 adipocyte differentiation [80].

Consistent findings demonstrated increased Akt phosphorylation following GLP−1 incubation (10^{-7} M) during the early phase of 3T3-L1 cell differentiation (first 24 h). In contrast, during the late phase (day 8), adipogenic markers, including Ppar-γ, C/Ebp-α, Lpl, and Fabp4, were upregulated at both mRNA and proteins levels [81].

In addition, GLP−1 modestly affected lipid droplet accumulation, as evidenced by a reduction in lipid droplet size accompanied by an increase in droplet number at day 8 of differentiation under 10^{-7} M GLP−1 stimulation [81].

Further evidence indicates that liraglutide enhances 3T3-L1 adipogenesis in a dose-dependent manner (10^{-9}–10^{-7} M), as demonstrated by increased *Fabp4* and *Ppar-γ* expression at both mRNA and protein levels following 6–48 h of stimulation. This effect was accompanied by a dose-dependent downregulation of fatty acid synthase (Fasn), mediated through activation of PkA and mitogen-activated protein kinase (Mapk) signaling pathways.

Collectively, these findings suggest that liraglutide may promote adipocyte differentiation by modulating Mapk signaling pathways, which functions as a molecular switch driving the transition of preadipocytes toward terminally differentiated adipocytes in mice [82].

Liraglutide-induced adipogenesis and adipocyte differentiation have also been demonstrated in primary chicken-derived adipocytes, as reported by Zhang et al. who used 10^{-7} M liraglutide at the 4th, 6th, and 8th day of differentiation [83]. Liraglutide promoted adipocyte differentiation through overexpression of key markers of mature adipocytes, including enzymes involved in lipogenesis, like acetyl-CoA carboxylase (*Acc*) and *Fasn*, as well as regulatory factors, including *Ppar-γ*, *sterol regulatory element binding protein* (*Srebp*) *−1*, and *C/Ebp-α* [83].

Interestingly, El Bekay et al. [84] provided mechanistic evidence on GLP−1-mediated human adipocyte differentiation and lipid metabolism. *In vitro* stromal vascular fraction cells isolated from adipose tissue of obese T2DM patients were exposed to GLP−1 (10^{-8} M) during 15 days of differentiation, resulting in a significant downregulation of *adipocyte differentiation-related protein* (*ADRP*) and *FABP4* expression. Conversely, GLP−1 exposure increased the expression of *HSL* and *PLIN* genes.

In mature human adipocytes, GLP−1 (10^{-7} M, 12–24 h) also downregulated adipogenic and lipogenic markers (*PPARγ*, *SREBP−1*, *FABP4*, and *FASN*), while increasing lipolytic proteins (ATGL, phosphorylated hormone sensitive lipase, or HSL, and PLIN), as well as adiponectin at both mRNA and protein levels.

Moreover, in differentiated 3T3-L1 adipocytes, incubation with GLP−1 (10^{-7} M, 24 h) reduced cytosolic Ca^{2+} levels (~13%), supporting a role for Ca^{2+}-dependent signaling in addition to the canonical cAMP/PKA pathway. Notably, these effects were only partially inhibited by the GLP−1R antagonist exendin(9–39), suggesting the involvement of additional receptor mechanisms [84].

Interestingly, genes involved in glucose metabolism were not significantly affected by GLP−1 treatment, suggesting that its primary effects are directed toward adipocyte differentiation and lipid metabolism (e.g. lipogenesis and lipolysis) [84]. This aspect will be discussed in detail in 2.2. The findings of the aforementioned studies are summarized in Table 2 and Fig. 2.

2.1.3. Summary and suggestions for further studies

Current literature on the effects of GIP, GLP−1, and related agonists has primarily focused on *in vitro* models, largely employing murine-derived preadipocytes, differentiated in the presence of GIP or GLP−1, or respective agonists [74,79,83].

In alternative experimental settings, preadipocytes were first differentiated using standardized adipogenic cocktails and subsequently exposed to incretins [75,76,79,80]. Other studies performed adipocyte differentiation in the continuous presence of GIPR [77,78] or GLP−1R agonists [81,82].

Given the substantial heterogeneity in the composition and timing of adipogenic induction protocols, these findings should be interpreted with cautions when assessing the direct effects of GIP and GLP−1 signaling on adipocyte differentiation.

In addition to variability in cell culture systems and experimental conditions, the majority of currently available studies have been conducted using murine-derived preadipocytes. Evidence from human-based models remains limited and is mainly derived from studies employing SGBS cells [78] or primary adipocytes isolated from both subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) depots [84].

Therefore, further studies are required to better delineate the mechanistic pathways underlying the effects of GIPR, GLP−1R, and their agonists on adipocyte differentiation.

An additional, critical aspect concerns the choice of cellular models. Primary adipocytes are limited by restricted availability and reproducibility, short-term viability in culture, and substantial interindividual and depot-specific variability. In contrast, immortalized or tumor-derived human cell lines represent more reproducible and accessible *in vitro* systems and may provide valuable platform to investigating incretin-mediated adipocytes differentiation and downstream signaling pathways in humans [85–87].

2.2. Lipid metabolism

2.2.1. Brief introduction to lipogenesis and lipolysis regulators in adipocytes

In adipocytes, lipogenesis and lipolysis, processes responsible for lipid synthesis and breakdown, respectively, play a central in systemic energy and lipid homeostasis [88]. These processes are tightly regulated by distinct hormonal and metabolic stimuli and involve partially overlapping but functionally opposing regulatory networks.

Lipogenesis leads to triglyceride synthesis from FFAs and glucose (via *de novo* lipogenesis) and is predominately activated in fed state. Newly synthesized triglycerides are stored within intracellular lipid droplets in adipocytes.

Among positive regulators of lipogenesis, insulin plays a pivotal role

Table 2
Summary of experimental evidence of glucagon-like peptide−1 (GLP−1), glucose-dependent insulinotropic polypeptide (GIP), and their agonists on adipocyte differentiation.

Experimental model	Treatment	Results	Reference
In vivo			
• HFD-fed mice	100 μg/kg liraglutide twice daily for the final 17 days	• Liraglutide promoted adipogenesis	[79]
In vitro			
• 3T3-L1 preadipocytes	10^{-11} M, 10^{-10} M, and 10^{-9} M GIP for 2 h	• ↑ protein expression and activity of Lpl	[74]
• 3T3-L1 preadipocytes	10^{-8} M GIP for 15 min	• No effects on intracellular cAMP accumulation	[75]
• 3T3-L1 differentiated adipocytes	10^{-8} M GIP for 15 min	• ↑ intracellular accumulation of cAMP via Gipr activation	[75]
• 3T3-L1 preadipocytes	2×10^{-9} M GIP during the first 4 days of adipocyte differentiation	• GIP enhanced insulin-stimulated Fabp4 protein expression	[76]
• 3T3-L1 preadipocytes	2×10^{-8} M GIP 4-day exposure	• ↑ Glut4 translocation and glucose uptake	[76]
• Differentiated primary human and 3T3-L1 preadipocytes	2×10^{-10} M, 2×10^{-9} M, 2×10^{-8} M, 2×10^{-7} M GIP for 10 min	• ↑ activation of Akt in a dose-dependent manner	[76]
• 3T3-L1 preadipocytes	10^{-8} M and 10^{-7} M GIP for 7 days	• GIP alone failed to increase lipid accumulation • GIP enhanced insulin-stimulated lipid accumulation • GIP and insulin synergistically induced 3T3-L1 preadipocyte differentiation	[77]
• SGBS differentiated adipocytes	10^{-11} M−10^{-6} M GIP for 12 min	• Higher intracellular cAMP accumulation via GIPR activation in differentiated SGBS adipocyte compared to undifferentiated ones	[78]
• 3T3-L1 preadipocytes	10^{-9} M−10^{-7} M liraglutide	• ↑ preadipocyte proliferation and ↓ apoptosis via GLP−1R-dependent activation of Erk1/2, Pkc, and Akt signaling	[79]
• 3T3-L1 preadipocytes	10^{-8} M and 5×10^{-8} M GLP−1 up to 4 days	• ↑ protein expression of Wnt4, increasing the expression of adipogenic transcription factors	[80]
• 3T3-L1 preadipocytes	10^{-7} M GLP−1 during the first 8 days of adipocyte differentiation	• ↑ Akt phosphorylation during the first 24 h of differentiation • ↑ gene and protein expressions of Ppar-γ, C/Ebp-α, Lpl, and Fabp4 • ↑ number of lipid droplets and slightly ↓ of their size	[81]
• 3T3-L1 preadipocytes	10^{-9} M−10^{-7} M liraglutide from 6 up to 48 h	• ↑ gene and protein expressions of Fabp4 and Ppar-γ • ↓ Fasn expression via Pka and Mapk activation	[82]
• Primary chicken-derived adipocytes	10^{-7} M liraglutide at the 4th, 6th, and 8th differentiation days	• ↑ gene expression of *Acc, Fasn, Ppar-γ, Srebp−1,* and *C/Ebp-α*	[83]
• SVFCs explanted from adipose tissue of obese T2DM patients	10^{-8} M GLP−1 during 15 days of adipocyte differentiation	• ↓ gene expression of *ADRP* and *FABP4* • ↑ gene expression of *HSL* and *perilipin*	[74]

Abbreviations: ACC, acetyl-CoA carboxylase; ADRP, adipocyte differentiation-related protein; AKT, protein kinase B; cAMP, cyclic adenosine monophosphate; C/EBP-α, CAAT enhancer binding protein-α; ERK1/2, extracellular signal-regulated kinase 1/2; FABP4, adipocyte fatty acid-binding protein 4; FASN, fatty acid synthase; GIP, glucose-dependent insulinotropic polypeptide; GIPR, GIP receptor; GLP−1, glucagon-like peptide−1; GLP−1R, GLP−1 receptor; GLUT4, glucose transporter 4; HFD, high-fat diet; HSL, hormone sensitive lipase; LPL, lipoprotein lipase; MAPK, mitogen-activated protein kinase; PKA, protein kinase A; PKC, protein kinase C, PPAR-γ, peroxisome proliferator-activated receptor-γ; SGBS, Simpson-Golabi-Behmel syndrome; Srebp−1, sterol regulatory element binding protein−1; SVFC, stromal vascular fraction cell; T2DM, type 2 diabetes mellitus.

by promoting glucose uptake through the upregulation and translocation of GLUT4, as well as by inducing the expression of key lipogenic enzymes, such as ACC and FASN [88].

Conversely, lipogenesis is suppressed during fasting, a state in which lipid mobilization is favored and regulated by hormonal factors such as GH, leptin, and glucagon (GCG). These hormones promote lipolysis by enhancing the expression and activation of key lipolytic enzymes, including patatin-like phospholipase domain-containing protein 2 (PNPLA2) and ATGL.

ATGL interacts with its activator α/β-hydrolase domain-containing protein 5 (ABHD5), which is released from PLIN−1 on the surface of lipid droplets, thereby enabling the hydrolysis of stored triglycerides and the mobilization of energy reserves [89,90].

Among the positive regulators of lipolysis, GH signaling promotes lipid mobilization through activation of the MEK/ERK signaling pathway, which contributes to PPAR-γ inhibition and HSL phosphorylation, ultimately enhancing lipolytic activity [91].

Leptin functions both centrally and peripherally as a key regulator of energy storage, modulating appetite, energy expenditure, and lipid metabolism [92]. At the adipocyte level, leptin binding to its receptor activates intracellular signaling cascades that promote lipid oxidation and triglyceride hydrolysis, thereby enhancing lipolysis while concurrently inhibiting lipogenesis [92,93].

Conversely, GCG counteracts insulin action by suppressing lipogenesis and promoting lipolysis, primarily through cAMP-dependent signaling pathways that stimulate lipolytic enzyme activity [94].

At the transcriptional level, lipogenesis is regulated by a network of key transcription factors, including SREBP−1c, carbohydrate-responsive-element-binding protein (ChREBP), and PPARγ. ChREBP is primarily activated by glucose and plays a central role in de novo lipogenesis [95], whereas SREBP−1c acts as a master regulator of genes encoding enzymes required for triglyceride synthesis [96]. PPARγ promotes lipid accumulation by coordinating adipogenic and lipogenic gene expression, including the modulation of lipolytic components such as [96].

Importantly, the activity of these transcriptional modifications, including phosphorylation, SUMOylation, and O-GlcNAcylation, which can shift their function output toward either lipogenesis or lipolysis [88].

Moreover, liver X receptors (LXR)-α, a nuclear hormone receptor activated by oxysterols and insulin, also contributes to the upregulation of lipogenic gene expression [97]. Overexpression of LXRα in 3T3-L1 preadipocytes has been shown to enhance glucose uptake, further supporting its role in coordinating lipid and glucose metabolism [98].

Similarly, upstream stimulatory factor 1 (USF1), an insulin-responsive transcription factor, participates in the activation of lipogenic genes. However, specific genetic polymorphisms may impair USF1 responsiveness to insulin, potentially shifting cellular metabolism toward increased lipolytic activity [99,100].

2.2.2. Evidence on modulation of lipid metabolism by GIP, GLP−1, and their agonists

Given the incretin nature of GIP and GLP−1 and their systemic regulatory role, elucidating their effects on lipid metabolism, particularly at the levels of adipocytes, is of primary importance. A growing body of evidence identified GIP and GLP−1 as key modulators of adipocyte lipid metabolism.

Specifically, GIP has been associated with insulin-like effects,

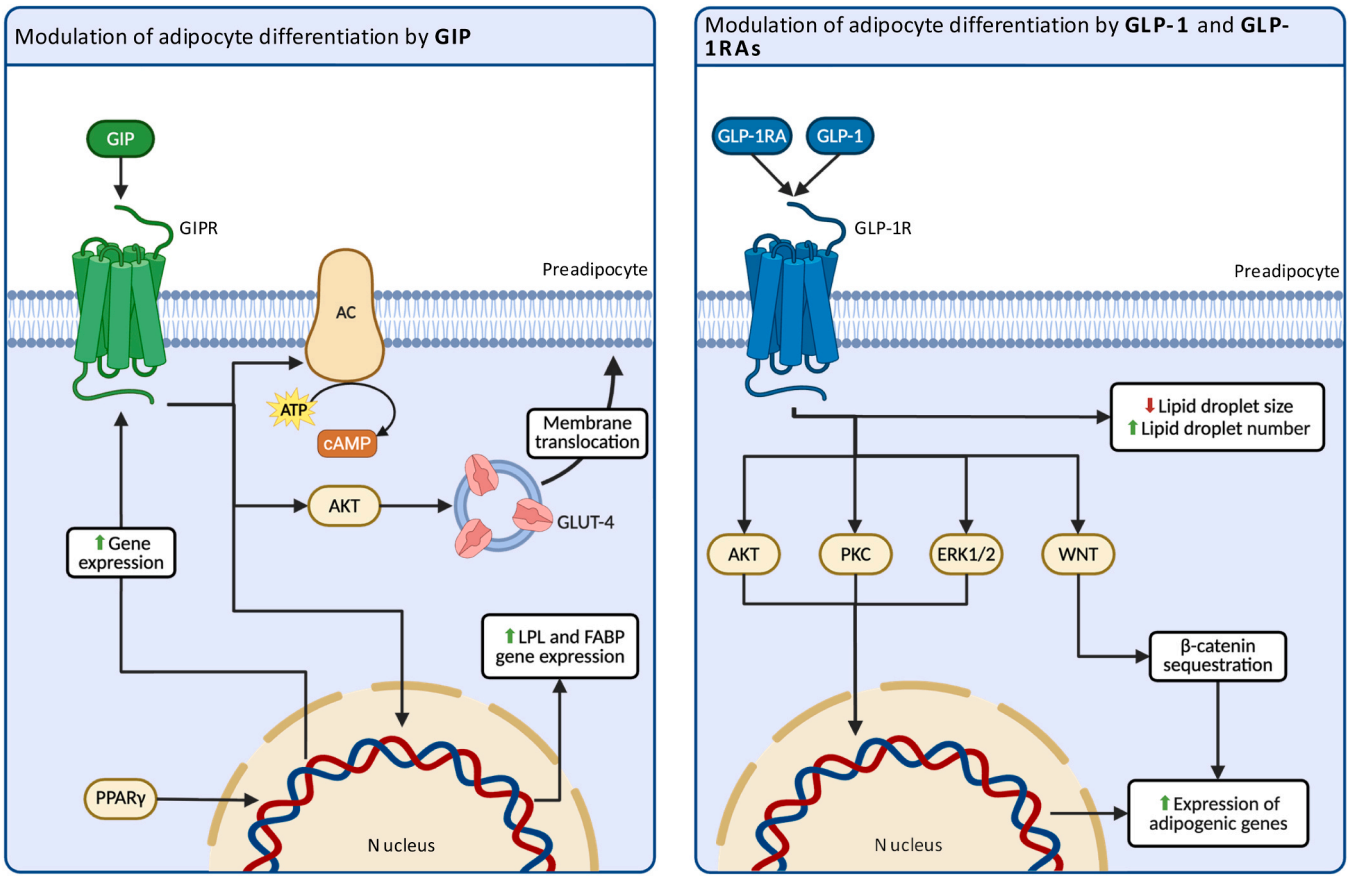

Fig. 2. Modulation of adipocyte differentiation by GIP, GLP−1, GLP−1 receptor agonists. *GIPR* gene expression is promoted by PPARγ activity and increases during late stage preadipocyte differentiation. GIP stimulates adenylate cyclase increasing cAMP production by binding GIPR. GIPR activation also induces AKT phosphorylation that in turn promotes GLUT−4 membrane translocation and *LPL* and *FABP* gene expression. Both GLP−1 and GLP−1RAs are able to induce different signaling pathways including AKT, PKC, and ERK1/2 that regulate the expression of genes involved in adipogenesis in preadipocytes. Similarly, GLP−1R activation leads to WNT signaling promoting β-catenin sequestration and, consequently, expression of adipogenic genes. GLP−1 and GLP−1RAs can also regulate lipid droplets number and size, promoting the development of multiple small lipid droplets. ***Abbreviations*:** AKT, protein kinase B; cAMP, cyclic adenosine monophosphate; ERK1/2, extracellular signal-regulated kinase 1/2; FABP, fatty acid binding protein; GIP, glucose-dependent insulinotropic polypeptide; GIPR, GIP receptor; GLP−1, glucagon-like peptide; GLP−1R, GLP−1 receptor; GLP−1RA, GLP−1R agonist; GLUT−4, glucose transporter−4; LPL, lipoprotein lipase; PKC, protein kinase-C; PPARγ peroxisome proliferator activated receptor γ; WNT, wingless-related integration site.

promoting lipid synthesis and storage in response to food intake [101]. Using differentiated murine 3T3-L1 adipocytes, primary human subcutaneous adipocytes, and the Vancouver diabetic fatty Zucker rat model, exposure to GIP (10^{-7} M for 24 h) in the presence of insulin (1 nM) resulted in a 2.6-fold increase in LPL activity and triglyceride accumulation in 3T3-L1 adipocytes. These effects were mediated by activation of protein kinase B (PKB) and dephosphorylation of AMP-activated protein kinase (AMPK) [102].

Notably, comparable effects were not observed following GLP−1 treatment (10^{-7} M for 24 h) in the presence of insulin (1 nM) in differentiated 3T3-L1 adipocytes. Consistently, in human-derived subcutaneous primary adipocytes, GIP (10^{-7} M for 24 h), but not GLP−1, under identical conditions, increased LPL activity and triglyceride storage [102].

In a subsequent study, the same group confirmed that GIP (10^{-7} M up to 24 h) induced an approximately 3-fold increase in LPL expression and activity in insulin-stimulated human adipocytes, whereas GLP−1 again failed to elicit comparable effects [103].

In vivo, a 2-week GIP infusion (10 pmol/kg/min) increased Lpl activity and PkB phosphorylation, while reducing Ampk phosphorylation in epididymal adipose tissue of Vancouver diabetic fatty Zucker rats, ultimately promoting triglyceride storage [102].

Interestingly, mice fed a HFD from 7 to 50 weeks of age exhibited greater accumulation of both subcutaneous and visceral fat compared with HFD-fed *Gipr*$^{-/-}$ mice, suggesting a role for GIPR signaling in the regulation of adipose tissue expansion and lipogenesis [46].

At the cellular level, GIP stimulation (10^{-7} M for 30, 60, and 240 min), in combination with insulin (0.2 nM), enhanced insulin-induced GLUT4 translocation, consistent with increased glucose uptake in differentiated 3T3-L1 adipocytes. This effect was observed in cells expressing either wild-type GIPR or the E354Q GIPR variant associated with insulin resistance and T2DM [104].

Enhanced glucose uptake was also observed in isolated rat adipocytes derived from epididymal fat of *ad libitum*-fed rats, following GIP exposure (10^{-8} M for 45 min) in the presence of insulin (0.2 ng/mL) [105]. Interestingly, in the same experimental model, GIP alone (10^{-8} M for 90 min) exerted lipolytic effects, as indicated by increased glycerol release in the cell culture medium compared to basal conditions [105]. However, when administered after stimulation with GCG (3×10^{-9} M) or isoproterenol (2×10^{-7} M), both established lipolytic agents, GIP reduced glycerol release, suggesting an inhibitory effect on stimulated lipolysis [105].

Notably, GIP has also been shown to exert lipolytic effects in differentiated murine 3T3-L1 adipocytes (10^{-11}–10^{-6} M for 4 h) [106] and in primary rat adipocytes (10^{-8} M for 1 h) [107], as evidenced by increased release of FFAs and glycerol, along with elevated intracellular cAMP levels. Interestingly, these effects were observed under GIP alone, whereas co-exposure to GIP (10^{-8} M for 4 h) and insulin (10^{-8} M)

abolished the lipolytic response in differentiated 3T3-L1 adipocytes, indicating that insulin counteracts GIP-induced lipolysis [106].

More recently, Yu et al. [108] demonstrated that adipose tissue-specific GIPR expression in transgenic obese mice fed with doxycycline- HFD (50 or 600 mg/kg for up to 12 weeks) was associated with weight loss and enhanced and lipolysis, suggesting a role for GIPR in adipose tissue metabolic remodeling. Mechanistically, GIPR activation promoted lipid oxidation, increased energy expenditure, and stimulated thermogenesis in the same experimental models and from subcutaneous white adipose tissue (WAT)-derived adipocytes, through SERCA-mediated activation of the futile calcium cycle [108]. Moreover, these transgenic obese mice, specifically over-expressing GIPR in the adipose tissue, showed lower food intake and appetite suggesting that adipose-specific GIPR signaling may also exert systemic metabolic effects [108].

In contrast, the effects of GLP−1 on lipid metabolism have been extensively investigated since its discovery in 1983 [109]. GLP−1 has attracted considerable attention for its regulatory role in lipid and cholesterol metabolism, with implications for the occurrence and development of metabolic and cardiovascular diseases [110].

Particularly, GLP−1 modulatory functions on lipid metabolism have been investigated in epididymal adipocytes isolated from control versus streptozotocin-induced diabetic rats [111]. GLP−1 (10^{-9} M) stimulated glucose uptake via activation of Pi3k and Mapk pathways, although less effectively than insulin under control conditions. In contrast, in adipocytes from diabetic rats, GLP−1-induced glucose uptake exceeding that of insulin, likely reflecting insulin resistance, and was primarily mediated through PkB activation [111]. Of note, GLP−1 positively modulated both lipogenic and lipolytic effects via activation of Mapk and PkC with comparable efficacy in both control and diabetic rats, compared to insulin [111].

Within adipogenesis-focused studies, the GLP−1R agonist liraglutide has been shown to exert stage-dependent effects on lipid metabolism. In 3T3-L1 preadipocytes, liraglutide promotes lipogenesis and lipid accumulation, whereas in mature adipocytes it exerts inhibitory effects [72]. Specifically, in differentiated 3T3-L1 adipocytes, 24-hour exposure to liraglutide (10^{-7} M) led to Glp−1r-mediated downregulation of *Fasn* expression through activation of Pka, Mapk (*e.g.*, Erk1/2), and Creb signaling pathways [82] Consistently, in vivo liraglutide administration (300 μg/kg/day for 4 weeks) in HFD–fed ob/ob mice reduced VAT mass and body weight, supporting its anti-lipogenic effects in mature adipose tissue [82].

Furthermore, liraglutide (10^{-7} M for 2 h) was shown to modulate lipid metabolism in chicken-derived adipocytes by inhibiting *de novo* lipogenesis. His effect was evidenced by downregulation of *Acc*, *Fasn*, *Ppar-γ*, *C/Ebp-α*, and *Srebp−1* mRNA expression, alongside increased phosphorylation of Ampk (p-Ampk) and Acc (p-Acc). Overall, these changes suggested a limited intracellular triglyceride and lipid droplet accumulation.

In recent years, increasing attention has been directed toward TZP, a dual GIPR/GLP−1R agonist, which has been shown superior efficacy in reducing body weight and circulating triglyceride levels in T2DM patients compared with selective GLP−1RAs [112,113]. These enhanced effects have been partly attributed to sustained GIPR activation.

Consistently, studies in both murine and human-derived subcutaneous adipocytes have shown that TZP (10^{-12}–10^{-6} M for 2 h) increases glucose uptake, thereby fueling glycerol synthesis and subsequent triglyceride formation and storage, both in the presence or absence of insulin (100 nM–10 fM) [114]. Moreover, improved lipid clearance, particularly increased fatty acid uptake into adipocytes has been observed in diet-induced obese mice treated with a long-acting GIPR agonist (LAGIPRA, 48 pmol/kg/min for 14 days) and in human primary adipocytes exposed to TZP (10^{-12}–10^{-6} M for 30–90 min) in the presence of insulin (100 nM), without a concomitant increase in adiposity. Collectively, these findings suggest that dual GIPR/GLP−1R agonism enhances post-prandial lipid handling, reducing circulating triglyceride levels and improving glycemic control over time, likely through sustained GIPR activation. [114] The main findings of these studies are summarized in Table 3 and Fig. 3.

2.2.3. Summary and suggestions for further studies

The heterogeneous effects of GIP on adipocyte lipid metabolism likely reflect differences in experimental conditions, including hormone concentration, duration of exposure, and the presence or absence of insulin, as well as the use of in vitro versus in vivo models [115,116]. These variables make complex finding interpretations and limit direct translation from preclinical to clinical outcomes. In addition, intercellular variability in GIPR expression across human adipocyte subsets represents a critical factor, as GIPR distribution is not uniform and may influence the responsiveness to GIP or GIP-related agonists [108].

Similarly, GLP−1R expression is relatively low in human adipocytes, and its variability among adipocyte populations may further affect the translational relevance of preclinical data [117].

Despite promising preclinical evidence on dual GIPR/GLP−1R agonists, several limitations should be considered. Notably, species-specific differences in receptor pharmacology may impact translational validity. Indeed, TZP was designed based on the human GIP sequence, which differs by approximately 20% from the mouse counterpart, potentially resulting in reduced GIPR activation in mouse models [118]. Moreover, differential activation profiles of GLP−1R and GIPR between mouse and human systems - demonstrated, for instance, in pancreatic islet β-cells - highlight the need for further investigation in human adipocyte models [119]. Such dynamics remain to be further clarified in human-derived adipocytes.

2.3. Insulin resistance and glucose uptake

2.3.1. Brief introduction to main mechanisms regulating insulin sensitivity

Insulin coordinates an integrated anabolic response to nutrient availability by binding to its receptor (IR) on the plasma membrane of target cells [120]. Althoug h IR is ubiquitously expressed, insulin-mediated regulation of glucose homeostasis primarily occurs in skeletal muscle, liver, and WAT. Under physiological conditions, insulin suppresses lipolysis while promoting glucose uptake and lipogenesis in WAT, thereby contributing to glycemic controls and facilitating lipid clearance from the circulation [121].

By contrast, in a systemic dysmetabolic state, WAT and other insulin responsive organs become progressively less sensitive to insulin, leading to insulin resistance. This establishes a vicious cycle in which compensatory hyperinsulinemia develops yet fails to effectively promote glucose uptake and lipid clearance in target tissues [121].

Recent evidence highlights depot-specific differential gene and protein expression of key insulin signaling components, in morbidly obese (MO) individuals with type 2 diabetes (T2DM) or hyperinsulinemia [122]. These include the PI3K regulatory subunit p85 (p85PI3K), the endocytic trafficking protein Rab5, and its guanine nucleotide exchange factor Gapex5. Mechanistically, p85PI3K participates in the activation of the PKB/AKT signaling cascade upon recruitment to insulin receptor substrate−1 (IRS−1), a process facilitated by Rab5, ultimately promoting GLUT4 translocation to the plasma membrane [123]. Gapex5, in turn, enhances Rab5 activity by stabilizing its GTP-bound active form [124]. In this context, Mourelatou et al. reported deregulated expression of RAB5 and GAPEX5, along with increased p85PI3K mRNA levels in subcutaneous abdominal adipose tissue from MO/T2DM subjects. These alterations are consistent with impaired glucose uptake and increased lipid accumulation, potentially contributing to insulin resistance [122]. Moreover, a positive correlation between p85PI3K mRNA expression in VAT and the homeostatic model assessment for insulin resistance (HOMA-IR) index in hyperinsulinemic patients suggests a link with progressive metabolic dysfunction and the development of T2DM. [122]

Insulin resistance is increasingly recognized as a systemic disorder in which dysfunctional adipose tissue plays a central role in driving whole-

Table 3
Overview of experimental evidence of glucagon-like peptide−1 (GLP−1), glucose-dependent insulinotropic polypeptide (GIP) and their agonists on lipid metabolism.

Experimental model	Treatment	Results	Reference
In vivo			
• Vancouver diabetic fatty Zucker rats	10 pmol/kg·min GIP for 2 weeks	• ↑ Lpl activity in epididymal fat • ↑ p-PKB and ↓ p-AMPK levels in epididymal fat • ↑ triglyceride content in epididymal fat	[102]
• HFD -fed mice	GIP	• ↑ Subcutaneous and visceral fat deposition	[46]
• Transgenic obese mice fed with Dox-HFD expressing GIPR in the adipose tissue	GIPR over-expression induced by Dox	• ↑ lipid oxidation, ↑ energy expenditure ↑ thermogenesis via SERCA-mediated calcium cycle • ↓ Food intake and appetite	[108]
• *ob/ob* mice fed with HFD	300 μg/Kg/day for 4 weeks liraglutide	• ↓ visceral adipose tissue and ↓ body weight	[82]
• DIO mice	48 pmol/kg/min LAGIPRA for 14 days	• ↑ Fatty acid uptake and ↑ lipid clearance without adiposity gain	[114]
In vitro			
• 3T3-L1 differentiated preadipocytes	10^{-7} M GIP for 24 h + 1 nM insulin	• ↑ Lpl activity • ↑ triglyceride storage via PkB activation and dephosphorylation of p-Ampk	[102]
• 3T3-L1 differentiated preadipocytes	10^{-7} M GLP−1 for 24 h + 1 nM insulin	• No significant effects on Lpl activity, PkB, or Ampk phosphorylation	[102]
• Human subcutaneous primary adipocytes	10^{-7} M GIP for 24 h + 1 nM insulin	• ↑ LPL activity • ↑ triglyceride storage	[102]
• Human subcutaneous primary adipocytes	10^{-7} M GLP−1 for 24 h + 1 nM insulin	• No significant effects on LPL activity, PKB, or AMPK phosphorylation	[102]
• Human subcutaneous adipocytes	10^{-7} M GIP for 24 h + 1 nM insulin	• ↑ LPL expression and activity	[103]
• 3T3-L1 differentiated adipocytes	10^{-7} M GIP for 30–60–240 min + 0.2 nM insulin	• ↑ GLUT4 translocation and glucose uptake	[104]
• Epididymal fat of *ad libitum*-fed rats	10^{-8} M GIP for 45 min + 0.2 ng/mL insulin	• ↑ Glucose uptake	[105]
• Epididymal fat of *ad libitum*-fed rats	10^{-8} M GIP for 90 min	• ↑ Glycerol release in the cell culture medium (↑ lipolysis)	[105]
• Epididymal fat of *ad libitum*-fed rats	10^{-8} M GIP for 90 min after GCG (3×10^{-9} M) or isoproterenol (2×10^{-7} M)	• ↓ Glycerol release in the cell culture medium	[105]
• 3T3-L1 differentiated adipocytes	10^{-11} – 10^{-6} M GIP for 4 h	• ↑ lipolysis	[106]
• 3T3-L1 differentiated adipocytes	10^{-8} M GIP for 4 h + 10^{-8} M insulin	• No effect on lipolysis	[106]
• Rat primary adipocytes	10^{-8} M GIP for 1 h	• ↑ release of FFAs and glycerol • ↑ intracellular cAMP levels	[107]
• Rat epididymal adipocytes (explants from diabetic and control rats)	10^{-9} M GLP−1	• ↑ Glucose uptake via Pi3k/Mapk (explants from control) or PkB (explants from diabetic rats)	[111]
• 3T3-L1 differentiated adipocytes	10^{-7} M liraglutide for 24 h	• ↑ lipogenic and lipolytic signaling via Mapk and PkC • ↓ Fasn via Pka/Mapk/Creb signaling; • ↓ gene expression of *Acc, Fasn, Ppar-γ, C/Ebp-α*, and *Srebp−1*	[82]
• Chicken-derived adipocytes	10^{-7} M liraglutide for 2 h	• ↑ p-AMPK and ↑ p-Acc levels	[83]
• Human and mouse subcutaneous adipocytes	10^{-12} – 10^{-6} M TZP for 2 h ±100 nM – 10 fM insulin	• ↓ intracellular triglyceride and lipid droplet content • ↑ Glucose uptake • ↑ glycerol synthesis • ↓ triglyceride synthesis and storage	[114]
• Human subcutaneous adipocytes	10^{-12} – 10^{-6} M TZP for 30–90 min ±100 nM insulin	• ↑ Fatty acid uptake and ↑ lipid clearance without adiposity gain	[114]

Abbreviations: ACC, acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase; ATGL, adipose triglyceride lipase; cAMP, cyclic adenosine monophosphate; C/EBP-α, CCAAT/enhancer-binding protein-α; DIO, diet-induced obese; Dox, doxycycline; ERK, extracellular signal-regulated kinase; FASN, fatty acid synthase; FFAs, free fatty acids; GCG, glucagon; GIP, glucose-dependent insulinotropic polypeptide; GIPR, GIP receptor; GLP−1, glucagon-like peptide−1; GLUT4, glucose transporter type 4; HFD, high-fat diet; HSL, hormone-sensitive lipase; LAGIPRA, long-acting GIPR agonist; LPL, lipoprotein lipase; MAPK, mitogen-activated protein kinase; p-, phosphorylated; PKA, protein kinase A; PKB (Akt), protein kinase B; PI3K, phosphoinositide 3-kinase; PPAR-γ, peroxisome proliferator-activated receptor-γ; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase; SREBP−1, sterol regulatory element-binding protein−1; T2DM, type 2 diabetes mellitus; TZP, tirzepatide; WAT, white adipose tissue.

body metabolic impairment [125]. In this context, insulin-resistant dysfunctional adipose tissue is characterized by elevated lipolytic activity, resulting in chronic release of non-esterified fatty acids (NEFAs) or FFAs and lipid-derived intermediates including glycerol, diacylglycerols, fatty acyl-CoAs, and ceramides, alongside adipokines, and proinflammatory mediators [126,127]. In obesity, excessive NEFA flux from adipose tissue represents a key driver of insulin resistance by impairing glucose uptake [128] and promoting substrate competition with glucose oxidation in insulin-responsive tissues, particularly skeletal muscle. [129]

Further *in vitro* evidence from differentiated murine C2C12 myocytes and 3T3-L1 adipocytes exposed to insulin (100 nM, 0–30 min) demonstrated that PKC theta impairs insulin signaling by promoting serine 1101 phosphorylation of IRS−1, thereby inhibiting its tyrosine phosphorylation. These inhibitory modifications disrupt proximal insulin signaling and lead to downstream attenuation of the Akt pathway, which is essential for insulin-stimulated GLUT4 translocation to the plasma membrane [130].

Insulin resistance is further exacerbated by obesity-associated low-grade chronic inflammation, characterized by macrophage infiltration into adipose tissue and polarization toward a pro-inflammatory phenotype, with increased secretion of cytokines such as IL−6 and TNF-α [131]. These inflammatory mediators activate multiple signaling pathways, including nuclear factor kappa B (NF-κB), suppressor of cytokine signaling (SOCS) proteins, c-Jun-N-terminal kinase (JNK), toll-like receptor (TLR), and WNT signaling, which converge on key components of the insulin signaling cascade, thereby impairing insulin actions and amplifying metabolic dysfunction [131].

Insulin resistance has also been linked to impaired autophagy at the cellular level. Chronic ER stress activation, induced by obesity and high calorie intake, initially activates adaptive autophagy to facilitate the clearance of misfolded proteins [132]. However, sustained stress can

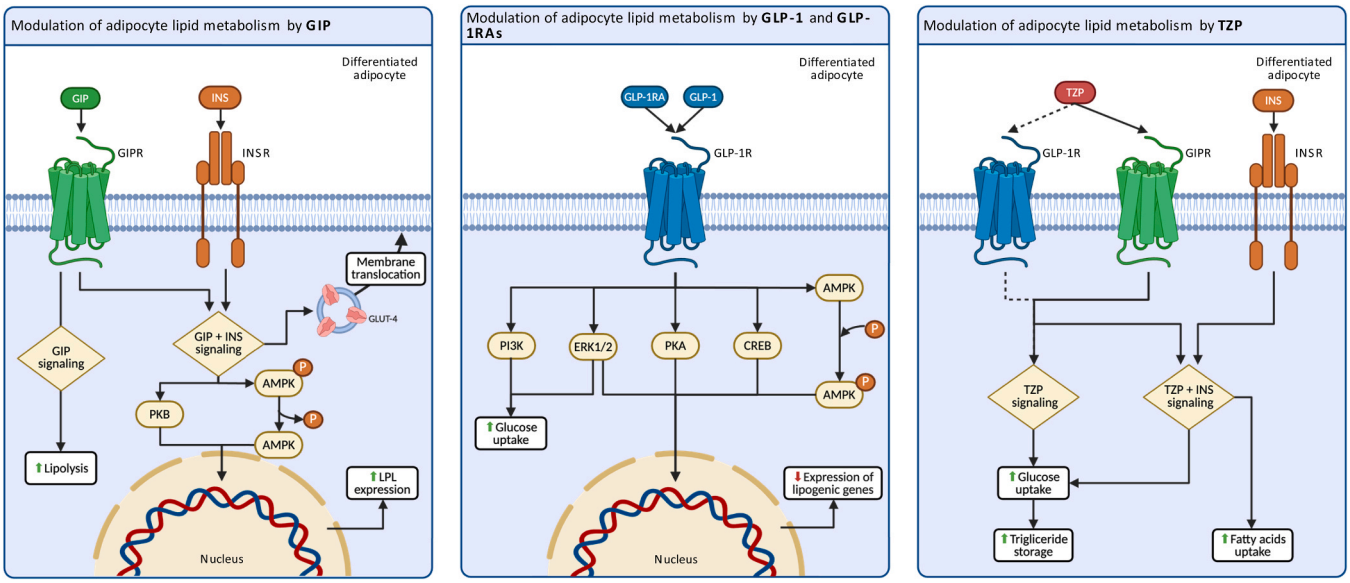

Fig. 3. Modulation of adipocyte lipid metabolism by GIP, GLP−1, GLP−1RAs, and TZP. GIPR activation promotes lipolysis. In presence of insulin, GIP stimulate LPL expression via PKB signaling and dephosphorylation of p-AMPK. GIP can also improve insulin-mediated GLUT−4 membrane translocation. GLP−1 and GLP−1RAs stimulate glucose uptake via PI3K and ERK1/2 signaling pathways. GLP−1R induces ERK1/2, PKA, CREB, and p-AMPK signaling pathways that are involved in the regulation of lipogenic gene expression. TZP promotes glucose uptake and in turn triglyceride storage by interacting with both GLP−1R and GIPR in presence and absence of insulin. TZP can also increase fatty acid uptake only in the presence of insulin. ***Abbreviations***: AMPK, AMP-activated protein kinase; CREB, cAMP responsive element binding protein 1; ERK1/2, extracellular signal-regulated kinase 1/2; GIP, glucose-dependent insulinotropic polypeptide; GIPR, GIP receptor; GLP−1, glucagon-like peptide; GLP−1R, GLP−1 receptor; GLP−1RA, GLP−1R agonist; GLUT−4, glucose transporter−4; INS, insulin; INSR, insulin receptor; LPL, lipoprotein lipase; p-AMPK, phosphorylated AMPK; PI3K, phosphoinositide 3-kinase; PKA, protein kinase-A; PKB, protein kinase B; TZP, tirzepatide.

compromise autophagic flux, leading to organelle dysfunction and the accumulation of damaged cellular components. This impairment promotes inflammation through activation of JNK/IκB kinase, which in turn activate serine kinases that phosphorylate IRS−1/2, thereby disrupting insulin signaling [133].

Notably, not all obese patients develop insulin resistance, likely reflecting underlying genetic and phenotype heterogeneity. Several mechanisms have been proposed to explain this variability, including difference in adipose tissue distribution, mainly subcutaneous rather than visceral depots, reduced ectopic fat accumulation (i.e., in liver and skeletal muscle), and a greater capacity for adipose tissue expansion via hyperplasia rather than hypertrophy [134]. In contrast, VAT is more prone to increased FFA release, contributing to ectopic fat accumulation and systemic lipotoxicity. These processes impair insulin signaling, including reduced GLUT4 translocation and diminished glucose uptake in insulin-responsive tissues [135].

Systemic lipotoxicity can also arise from the failure of subcutaneous adipose tissue to effectively store excess lipids, a process often linked to impaired adipogenesis in insulin-resistant obese patients [136]. Reduced adipogenic capacity is associated with pronounced adipose hypertrophy and activation of cell stress responses, including inflammation driven to pro-inflammatory mediators such as TNF-αα, IL−6, and MCP−1, as well as macrophage infiltration [137,138].

These alterations further compromise adipose tissue expandability and metabolic flexibility, promoting ectopic lipid deposition and exacerbating systemic insulin resistance. The critical role of inflammation in adipose tissue dysfunction will be discussed in 2.4.

2.3.2. Evidence on modulation of insulin sensitivity by GIP, GLP−1, and their agonists

Given the central role of insulin resistance in obesity and metabolic dysfunction, increasing attention has focused on the potential insulin-sensitizing effects of GIP and GLP−1 in peripheral tissues beyond their canonical actions on pancreatic β-cells [139,140].

Evidence from murine 3T3-L1 adipocytes differentiated with an adipogenic cocktail indicates that 24-hour exposure to GLP−1 or its analog exendin−4 (both at 10^{-8} M) does not significantly affect basal (insulin-independent) glucose uptake. In contrast, GLP−1 and exendin−4 enhance insulin signaling through modulation of key nodes of the pathway, including tyrosine/serine phosphorylation of Irβ, Irs−1, Akt, and glycogen synthase kinase−3ß (Gsk−3ß). This results in increased Glut4 expression and augmented glucose uptake and storage [141]. Interestingly, GLP−1 and exendin−4 were also able to restore insulin signaling, Glut4 expression, and glucose uptake in TNF-α–treated (insulin resistant) differentiated 3T3-L1 adipocytes in the presence of insulin, highlighting their capacity to counteract inflammation-induced insulin resistance [141].

Similarly, exendin−4 was shown to increase dose-dependent (10^{-9}–10^{-7} M) insulin-stimulated glucose uptake in differentiated 3T3-L1 adipocytes. Curiously, 10^{-7} M GLP−1 did not affect glucose uptake using the same experimental model in the presence of insulin [142].

Interestingly, liraglutide (10^{-7} M, 24-hour exposure) exerted insulin-sensitizing effects in differentiated 3T3-L1 adipocytes, as evidenced by increased expression and phosphorylation of Irs−1 and Akt, in the presence of insulin (100 nM for 20 min) [143]. In contrast, liraglutide treatment alone (10^{-7} M for 24 h) increased Akt expression without a corresponding increase in its phosphorylation levels, suggesting that liraglutide primarily enhances insulin-dependent activation of IR rather than directly it [143].

The aforementioned *in vitro* findings are supported by *in vivo* studies demonstrating that GLP−1R agonism improves insulin sensitivity through coordinated metabolic and anti-inflammatory mechanisms. In high-fat, high-sucrose diet-induced obese mouse models, liraglutide (200 μg/kg daily for two weeks) improved fasting glycemia, intraperitoneal glucose tolerance, and body weight, partly through reduced food intake [144]. In addition, liraglutide enhanced energy expenditure via activation of the Ampk-Sirtuin−1 (Sirt−1) signaling pathway in perigonadal adipose tissue. Specifically, liraglutide restored Ampk phosphorylation and Sirt−1 expression, both of which were suppressed by

the high-fat, high-sucrose diet [144].

Moreover, liraglutide-mediated GLP−1R activation has been shown to improve insulin sensitivity though attenuation of endoplasmic reticulum (ER) stress in visceral adipocytes from *ob/ob* (BKS.Cg-m+/+Leprdb/J) mice, exposed to 100 μg/mouse liraglutide for 2 weeks. Particularly, liraglutide induced consistent reduction of C/EBP Homologous Protein (CHOP), a pro-apoptotic mediator, and transcription factor activated in the presence of prolonged ER stress [145]. Mechanistically, *in vitro* studies further demonstrated that GLP−1 (5×10^{-8} M for 16 h) directly alleviates ER stress in differentiated visceral rat adipocytes by downregulating Chop expression and enhancing insulin signaling, as evidenced by increased Akt phosphorylation (Ser47) in thapsigargin-induced ER stressed differentiated visceral rat adipocytes [145]. Specifically, rat adipocytes were obtained from stromal fraction of rat VAT, differentiated with adipogenic cocktail, fasted for 6 h and thereby exposed to 5 μM thapsigargin (16 h), a known ER stress inducer [146], and finally exposed to 100 nM insulin (10 min). At the molecular level, GLP−1 reduced thapsigargin-mediated phosphorylation of Pancreatic/PKR-like ER kinase (PERK) and Eukaryotic initiation factor 2α (eIF2α) proteins, leading to a suppression of activating transcription factor (Atf) 4, a central mediator of CHOP-driven ER stress responses. Collectively, these findings indicate that GLP−1R activation mitigates ER stress–related signaling, thereby contributing to improved insulin sensitivity [145].

Evidence from thapsigargin-induced ER-stressed differentiated visceral rat adipocytes indicates that GLP−1 (5×10^{-8} M for 4 h) promotes autophagy, as demonstrated by overexpression of LC3-II, the lipidated membrane-bound form of the cytosolic microtubule-associated protein 1 A/1B-light chain 3, form I, LC3–1, and consequently increment of the LC3-II /LC3-I ratio [133,147]. These findings suggest that GLP−1R activation may ameliorate insulin resistance in ER-stressed adipocytes with disrupted autophagy via amelioration of ER stress and enhancing autophagy.

More recently, TZP has emerged as a potent modulator of insulin sensitivity. In HFD–induced insulin-resistant obese mice, TZP administration (10 nmol/kg/day for 14 days) improved insulin tolerance and metabolic parameters, including reductions in body weight, food intake, circulating leptin, triglycerides, free fatty acids, and both fed and fasting glucose and insulin levels These effects were accompanied by increased levels of insulin-sensitizing factors, such as adiponectin and insulin-like growth factor-binding protein 2 [148].

Similarly, TZP ameliorated insulin resistance by systemically increasing glucose infusion rate and glucose uptake in the same HFD-induced insulin-resistant obese mice. While the GLP−1R agonist semaglutide improved insulin resistance via weight-dependent mechanisms, TZP (3 nmol/kg) ameliorated insulin resistance both through weight-dependent and weight-independent mechanisms at a dose 3-fold lower than semaglutide (10 nmol/kg) [148].

Additional weight-independent insulin-sensitizing effects of TZP (10 nmol/kg for 14 days) were observed in Glp−1r$^{-/-}$mice fed a HFD for 12 weeks. In this model, TZP enhanced insulin-stimulated glucose disposal in WAT depots, supporting a GLP−1R–independent contribution - likely mediated by GIPR activation - to improve insulin sensitivity [148].

The main findings of the aforementioned studies are summarized in Table 4 and Fig. 4.

2.3.3. Summary and suggestions for further studies

Taken together, current literature indicates that GIP and GLP−1 signaling can modulate adipocyte dysfunction by potentiating insulin-dependent pathways governing glucose uptake, lipid turnover, and cellular stress responses. Nevertheless, the extent to which these effects represent direct receptor-mediated mechanisms *versus* secondary consequences of systemic metabolic improvement remains a subject of current evolving research [149].

Furthermore, much of the current knowledge has been derived from immortalized adipocyte cell lines or rodent models trying to recapitulate main obesity-related dysfunctional signatures and employing supra-physiological ligand exposure. However, these models poorly resemble effective pathophysiological mechanisms associated with obesity considering its prevalent monogenic induction in animal models compared to the polygenic and multifactorial nature, and complex molecular interplay of obesity occurring in humans [150,151].

Moreover, obesity-mimicking animal models may show strain-dependent pharmacological discrepancies in response to drugs [152,153]. Methodological pitfalls may also limit translatability to humans. For instance, diet-induced obese rodent models develop hyperinsulinemia but lack overt hyperglycemia, making them poor surrogates for studying obesity progression toward T2DM [144,154].

A key limitation of preclinical studies is that animal models of obesity are often used during their growth phase, complicating the interpretation of weight loss following pharmacological intervention, as observed changes may reflect attenuated weight gain rather true weight reduction [155]. In addition, environmental factors, such as diet composition (i.e., source of fats or carbohydrate content), can significantly influence metabolic phenotypes and the development of insulin resistance [156,157].

These limitations, together with the challenge of disentangling weight-loss–dependent effects from direct molecular mechanisms on adipocytes or their crosstalk with other insulin-responsive tissues, limit the translational relevance of preclinical findings.

Future investigations employing human-derived adipocytes, possibly integrated into more complex and physiologically relevant systems that incorporate additional components of the adipose tissue, will be essential to better recapitulate obesity-associated dysfunction and to define the pharmacodynamic effects of incretin-based therapies on adipocyte function and metabolic remodeling.

2.4. Adipokines, oxidative stress and inflammation

2.4.1. Brief introduction to adipokines, redox balance, and inflammation in functional and dysfunctional adipocytes

Adipose tissue functions as a metabolically active endocrine organ that plays a central role in the maintenance of metabolic homeostasis through the coordinated secretion of adipokines. In physiologically functional adipocytes, a balanced adipokine profile is maintained by insulin-sensitizing and anti-inflammatory mediators, such as adiponectin, together with preserved redox homeostasis [93,158].

The critical balance between adipokine production and release, including leptin, adiponectin, omentin, resistin, and visfatin was shown to be central in modulating not only oxidative stress, but also tissue and systemic inflammation [159–161]. *In vivo* and *in vitro* murine models have shown that adiponectin, one the major adipokines produced in the adipose tissue, can downregulate ER stress-mediated adipocyte apoptosis by upregulating *Ppar-α*, which in turn suppresses the expression of *Atf2*, involved in stress response [162]. Additionally, the adiponectin-driven activation of Ampk/Ppar-α signaling pathway has shown to be crucial for modulating inflammation and maintenance of health adipose tissue [162]. To this end, it has been shown the crucial balance between adiponectin and leptin release from adipocytes in modulating local (i.e., adipose tissue) and systemic functions, including energy metabolism, glucose homeostasis, and fat deposition [163]. In this regard, the adiponectin/leptin ratio has been mentioned among markers of dysfunctional adipose tissue [164]. Accordingly, its drop was observed in patients with metabolic syndrome, compared to increasing systemic inflammatory markers, such as C-reactive protein, and oxidative stress (i.e., levels of thiobarbituric acid reactive substances) [165]. Moreover, adiponectin levels were observed to drop in obesity and negatively correlate with body weight, favoring adipose tissue dysfunction [166]. Its drop was observed in patients with metabolic syndrome, compared to increasing systemic inflammatory markers, such as C-reactive protein and oxidative stress (i.e., levels of thiobarbituric acid reactive substances) [165]. Moreover, adiponectin levels were

Table 4
Summary of experimental evidence glucagon-like peptide−1 (GLP−1), glucose-dependent insulinotropic polypeptide (GIP), and their agonists on insulin resistance and glucose uptake.

Experimental model	Treatment	Results	Reference
In vivo			
• HFHSD-induced obese mice	200 μg/kg/day liraglutide for 2 weeks	• ↓ Fasting glucose; ↑ glucose tolerance; ↓ body weight via reduced food intake	[144]
• HFHSD-induced obese mice	200 μg/kg/day liraglutide for 2 weeks	• ↑ Energy expenditure via activation Sirt−1 pathway • ↑ AMPK phosphorylation and Sirtuin −1 protein expression	[144]
• ob/ob mice	100 μg/mouse liraglutide for 2 weeks	• ↓ ER stress and Chop protein expression • ↑ insulin sensitivity • ↑ insulin tolerance	[145]
• HFD-induced insulin-resistant obese mice	10 nmol/kg/day TZP for 14 days	• ↓ body weight and food uptake • ↓ serum levels of leptin, triglycerides, FFAs • ↑ serum levels of adiponectin and IGFBP2	[148]
• HFD-induced insulin-resistant obese mice	3 nmol/kg TZP vs 10 nmol/kg semaglutide	• ↑ insulin sensitivity via weight-dependent and independent mechanisms at lower dose compared to semaglutide	[148]
• Glp−1r$^{-/-}$mice fed with HFD	10 nmol/kg TZP for 14 days	• ↑ insulin-stimulated glucose disposal in WAT in a weight-independent manner	[148]
In vitro			
• Differentiated 3T3-L1 adipocytes	10^{-8} M GLP−1 or 10^{-8} M exendin−4 for 24 h	• No effect on glucose uptake	[141]
• Differentiated 3T3-L1 adipocytes	10^{-8} M GLP−1 or 10^{-8} M exendin−4 + insulin for 24 h	• ↑ phosphorylation of Irβ, Irs−1, Akt, Gsk−3β (↑ insulin signaling) • ↑ protein expression of GLUT4 • ↑ glucose uptake	[141]
• TNF-α–stimulated differentiated 3T3-L1 adipocytes	GLP−1 or exendin−4 + insulin	• Rescue of TNF-α–induced insulin resistance • ↑ GLUT4 expression and glucose uptake	[141]
• Differentiated 3T3-L1 adipocytes	10^{-9}–10^{-7} M Exendin−4 + insulin	• ↑ insulin-stimulated glucose uptake in a dose-dependent manner	[142]
• Differentiated 3T3-L1 adipocytes	10^{-7} M GLP−1 + insulin	• No significant effect on glucose uptake	[142]
• Differentiated 3T3-L1 adipocytes	10^{-7} M liraglutide for 24 h + 100 nM insulin for 20 min	• ↑ Irs−1 and Akt protein expression and phosphorylation	[143]
• Differentiated 3T3-L1 adipocytes	10^{-7} M Liraglutide for 24 h	• ↑ Akt protein expression without phosphorylation	[143]
• Thapsigargin-induced ER-stressed differentiated rat visceral adipocytes	5×10^{-8} M GLP−1 for 16 h + 100 nM insulin for 10 min	• ↓ Chop protein expression • ↑ Akt phosphorylation (improved insulin signaling)	[145]
• Thapsigargin-induced ER-stressed differentiated rat visceral adipocytes	5×10^{-8} M GLP−1 for 16 h	• ↓ PERK and eIF2α phosphorylation • ↓ Atf4 activation (↓ ER stress and improved insulin signaling)	[145]
• Thapsigargin-induced ER-stressed differentiated rat visceral adipocytes	5×10^{-8} M GLP−1 for 4 h	• ↑ LC3-II and LC3-II/LC3-I ratio levels (↑ autophagy activation)	[133]

Abbreviations: Akt, protein kinase B; AMPK, AMP-activated protein kinase; Atf4, activating transcription factor 4; CHOP, C/EBP homologous protein; ER, endoplasmic reticulum; eIF2α, eukaryotic initiation factor 2α; ERK1/2, extracellular signal-regulated kinase 1/2; FFAs, free fatty acids; GIP, glucose-dependent insulinotropic polypeptide; GLP−1, glucagon-like peptide−1; GLP−1R, glucagon-like peptide−1 receptor; GLUT4, glucose transporter type 4; Gsk−3β, glycogen synthase kinase−3β; HFD, high-fat diet; HFHSD, high-fat high-sucrose diet; IGFBP2, insulin-like growth factor-binding protein 2;Irβ, insulin receptor β subunit; Irs−1, insulin receptor substrate−1; LC3, microtubule-associated protein 1 light chain 3; PERK, Pancreatic/PKR-like ER kinase; SIRT1, sirtuin−1; TNF-α, tumor necrosis factor- α; TZP, tirzepatide; WAT, white adipose tissue.

observed to drop detrimentally in obesity and negatively correlate with body weight, favoring adipose tissue dysfunction [166].

In contrast, in obese patients, leptin has been mentioned as a major adipokine whose high circulating levels were associated with enhanced fat mass deposition peripherally and dysregulated appetite/satiety central circuits [167].

In adipocytes, leptin has been reported to exert pro-adipogenic effects [158], positively regulate lipolysis [168], and maintain a balanced inflammatory state by patrolling key pro-inflammatory cytokines [92]. Specifically, in murine preadipocytes, leptin was found to induce increased lipid droplet deposition, adipogenesis, lipogenesis, and inflammation via augmented production of pro-inflammatory cytokines, including IL−6, TNF-a, and IL−1β, supporting adipocyte dysfunction [169].

On the other hand, resistin, from “resistance to insulin”, was initially known to resist insulin action [170]. It is mainly produced by white adipocytes in rodents, whereas the main source of resistin in humans is represented by immune cells (i.e., macrophages) [171]. Currently, little is known about the pathophysiological role of resistin. Studies based on 3T3-L1 adipocytes showed that resistin secretion is stimulated by insulin, intracellular cAMP, and/or Ca^{2+} [172]. Moreover, resistin secretion was observed in mouse primary inguinal white adipocytes following β3-adrenergic receptor (Ar) activation. Its secretion, but not its production, appeared reduced in white adipocytes obtained from HFD-induced obese mice, suggesting impaired resistin secretory pathways in obese mice [172]. Interestingly, resistin was also co-localized with adiponectin in secretory vesicles from mouse primary inguinal white adipocyte, suggesting potential co-secretion within same vesicles [172]. In humans, resistin was showed to drive release of pro-inflammatory cytokines, including TNF-α, IL−1β, IL−6, IL−12 and IL−18 via activation of NF-κB, supporting its direct involvement in orchestrating inflammatory response, specifically in metabolic dysfunction and obesity [173,174]. Accordingly, resistin was found to induce overexpression of NF-κB in both human and murine macrophages [175]. Consistently, resistin induced expression of a wide variety of pro-inflammatory markers in human-derived WAT explants, further supporting its pro-inflammatory role in adipocyte dysfunction [176].

Visfatin is another adipokine proposed to be involved in obesity and inflammatory mechanisms associated with adipocyte dysfunction. Initially identified in B lymphocyte precursors cells, where acts as a growth differentiation factor [177], visfatin positively correlates with obesity and metabolic syndrome considering its high circulating levels [178,179]. Visfatin was found mainly expressed in visceral WAT, particularly in macrophages than in mature adipocytes derived from human explants, suggesting its potential involvement in inflammatory-dependent adipose tissue dysfunction [180]. However, poor evidence has been gathered about the pathophysiological mechanisms driven by visfatin in dysfunctional adipocytes, based on current literature.

Notably, metabolically dysfunctional adipocytes, in obesity and T2DM, were shown to increase the production and release of pro-inflammatory cytokines, including IL−6, TNF-α and IL−1β, in the

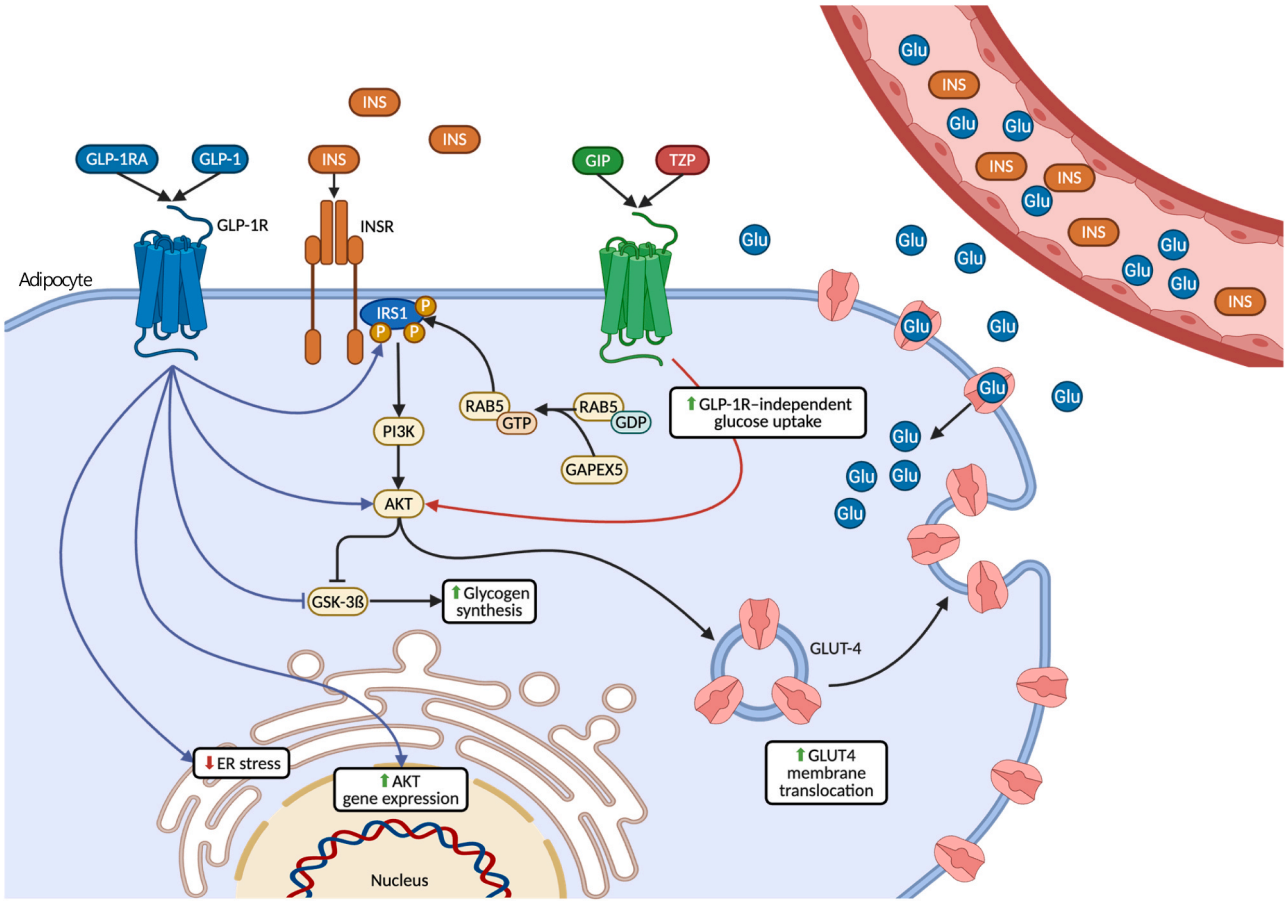

Fig. 4. Modulation of adipocyte insulin sensitivity by GIP, GLP−1, GLP−1RAs, and TZP. INSR activation promotes the recruitment and phosphorylation of IRS1. GAPEX5 stabilizes GTP-bound active form of RAB5 that in turn facilitates IRS1 recruitment. Phosphorylated IRS1 activates PI3K/AKT signaling leading to GLUT4 membrane translocation and glycogen storage by inhibiting GSK−3β. GLP−1 and GLP−1RAs enhance insulin signaling through multiple mechanisms, including phosphorylation of IRS1 and AKT, inhibition of GSK−3β, ER stress reduction, and increased AKT gene expression. GIP and TZP stimulate glucose uptake via GLP−1R-indipendent AKT activation. ***Abbreviations:*** AKT, protein kinase B; ER, endoplasmic reticulum; ERK1/2, extracellular signal-regulated kinase 1/2; GAPEX5, GTPase activating protein and VPS9 domains 1; GDP, guanosine diphosphate; GIP, glucose-dependent insulinotropic polypeptide; GIPR, GIP receptor; GLP−1, glucagon-like peptide−1; GLP−1R, glucagon-like peptide−1 receptor; Glu, glucose; GLUT4, glucose transporter type 4; GSK−3β, glycogen synthase kinase−3β; GTP, guanosine triphosphate; INS, insulin; INSR, insulin receptor; IRS1, insulin receptor substrate−1; PI3K,; RAB5, RAS-related protein RAB−5A; TZP, tirzepatide.

presence of hyperglycemia, overall contributing to systemic low-grade chronic inflammation [181–183].

Oxidative stress has also been proposed among molecular mechanisms involved in adipocyte functional derangements in obesity [184], including overnutrition-mediated mitochondrial stress and impaired antioxidant defense [185]. Evidence from experimental studies found oxidative stress boosting adipocyte proliferation, differentiation and size (hypertrophy) [186,187], leading to systemic metabolic derangements [188].

In addition, oxidative stress in metabolically deranged adipose tissue supports the activation of pro-inflammatory mechanisms, further promoting insulin resistance and low-grade chronic inflammation [189].

Taken together, these alterations have stimulated growing interest in endocrine pathways that regulate adipokine secretion, redox homeostasis, and inflammatory signaling in adipocytes, including incretin-mediated mechanisms involved in adipocyte functional remodeling in the context of obesity.

2.4.2. Evidence on modulation of adipokine expression, redox homeostasis, local and systemic inflammation by GIP, GLP−1, and their agonists

Accumulating evidence has highlighted that GIP and GLP−1 directly affect adipokine secretion, cellular stress responses, and inflammatory signaling within adipose tissue across experimental models. Accordingly, in obese C57BL/6 mice fed with a HFD, four-week treatment with semaglutide (40 μg/Kg once every 3 days) has been associated with improved adipose tissue metabolism and marked suppression of inflammation in VAT, including reductions in Tnf-α (−60%), Il−6 (−55%), Mcp−1 (−90%), and leptin (−80%) [190].

Similarly, in HFD–induced obese male C57BL/6 J mice, treatment with TZP (1.2 mg/kg, twice weekly for 12 weeks) reduced local adipose tissue inflammation. This effect was associated with decreased M1 macrophage infiltration, increased M2 macrophage polarization in visceral fat, and reduced expression of pro-inflammatory mediators, including Tnf-α, Il−6, Mcp−1, and interferon (Ifn)-γ [191].

In vitro studies using 3T3-L1 adipocytes have demonstrated that GLP−1 modulated adipokine signaling by inducing a dose- and time-dependent increase in visfatin secretion and gene expression through activation of the PKA pathway, with significant effects observed at concentrations as low as 10^{-10} M, maximal responses at 10^{-9} M, and sustained up to 24 h. Therefore, GLP−1–mediated visfatin upregulation supports redox homeostasis and attenuated local inflammatory responses by counteracting ER stress–induced suppression of visfatin [192].

Moreover, GLP−1 receptor agonist exendin−4 (2.5×10^{-9} M, administered for 8 h) further enhanced mRNA *adiponectin* expression in differentiated 3T3-L1 adipocytes, while simultaneously inhibiting pro-inflammatory cytokine mRNA expression, such as IL−6 and IL−18, suggesting anti-inflammatory effects [193].

Comparable effects have been observed in human adipose-derived stem cells (hADSCs) isolated from subcutaneous adipose tissues of obese individuals undergoing bariatric surgery. Treatment with the GLP−1 receptor agonist liraglutide (10^{-9}–10^{-7} M) has been shown to induce a 2.64-fold increase in adiponectin mRNA expression, indicating a shift toward a more insulin-sensitive adipokine profile [194].

The anti-inflammatory remodeling induced by GLP−1RAs was also observed systemically, given the reduction of major inflammation biomarkers, including high-sensitivity C-reactive protein (hs-CRP), in subjects with obesity and T2DM. To this regard, literature systematic review and meta-analysis have reported 44% reduction of CRP index in subjects with or without T2DM treated with semaglutide only, either given orally (3, 7, 14 or 50 mg daily) or subcutaneously (1, 1.7 or 2.4 mg once weekly), for at least 3 months with a follow up of 1 up to 3 years, according to the selected studies [195]. In a different systematic review and meta-analysis, it was also observed that GLP−1RAs, administered to subjects with T2DM with a follow up of 6 months, compared to oral diabetic medications and insulin led to the reduction of CRP, IL−6, TNF-α, IL−1β and leptin, while increased adiponectin levels [196]. The systemic anti-inflammatory effects of semaglutide in subjects with T2DM were also analyzed in SUSTAIN 3 (1 mg weekly, via subcutaneous injection for 56 weeks) and PIONEER 1 (3, 7 or 14 mg daily, via oral administration for 26 weeks), 2 (14 mg daily; oral administration for 52 weeks), and 5 (4 mg daily; oral administration for 26 weeks) randomized clinical trials by measuring hs-CRP. The hs-CRP ratios-to-baseline appeared significantly reduced following semaglutide compared to exenatide extended-release and empagliflozin treatments, although this effect was partially mediated (20.6–61.8%) by changes in glycated hemoglobin and body weight [197].

Overall, these findings suggest that GLP−1RAs may exert also direct, rather than only undirect, effects on systemic inflammation.

In contrast, GIP has been linked to proinflammatory responses in adipose tissue. In brown adipose tissue cells, *in vitro* exposure to 10^{-7} M [D-Ala^2]-GIP increased IL−6 mRNA expression and IL−6 secretion within 1–24 h, consistent with *in vivo* findings showing that acute administration of [D-Ala^2]-GIP (24 nmol/kg) rapidly elevated plasma IL−6 levels in wild type mice within 30 min to 2 h. Moreover, loss of GIPR signaling has been shown to alter basal expression of genes involved in inflammation, thermogenesis, and lipid utilization [198].

Similarly, *in vitro* adipocyte/macrophage co-culture studies using human-derived SGBS/monocyte-derived primary macrophages and murine 3T3-L1/RAW 264.7 cells have demonstrated that GIP-induced inflammation was dependent on adipocyte–macrophage crosstalk [199]. However, following prolonged GIP treatment (10 nmol/Kg daily for 1–4 weeks) no pro-inflammatory effects were detected in wild type mice, based on mRNA expression of Mcp−1 and Il−6 markers [200].

On the other end, clinical evidence has further supported a potential pro-inflammatory role of GIP in adipose tissue. Accordingly, in a randomized, single-blind study involving 17 healthy and slightly obese men, acute intravenous infusion of GIP at postprandial concentrations (2 pmol/kg/min) for 240 min was shown to increase pro-inflammatory adipokine expression in human subcutaneous adipose tissue, along with elevated circulating MCP−1 levels [183].

Curiously, anti-inflammatory effects on systemic inflammation have been reported mainly with the use of TZP. Accordingly, SUMMIT, a phase III clinical trial, reported a 37.2% reduction of the CRP in patients with Heart Failure with a Preserved Ejection Fraction and obesity following TZP treatment (from 2.5 mg, increased every 4 weeks, till 15 mg) for 52 weeks [201]. Similarly, in a retrospective cohort study, it was found a reduction from 3 to 2.24 mg/L of the hs-CRP levels in patients with T2DM and MASLD treated with 2.5 mg/week TZP for 6 months, after GLP−1RAs only (baseline hs-CRP values not specified) [202].

Interestingly, a phase II clinical trial in patients with T2DM, treated with 1, 5, 10 or 15 mg TZP once per week versus 1.5 mg dulaglutide or placebo for 26 weeks, observed a reduction of tissue/systemic inflammation, cellular stress and endothelial dysfunction markers. Particularly, 10 and 15 mg TZP significantly reduced chitinase−3 like-protein−1, intercellular adhesion molecule 1, leptin, and growth differentiation. While only 15 mg of TZP significantly reduced hs-CRP levels [203]. These findings suggest that TZP may exert protective effects also on the cardiovascular system by lowering common cardiovascular disease (CVD) risk factors.

In a different phase II clinical trial in patients with obesity only or obesity and T2DM treated with 140, 280 or 420 mg maridebart cafraglutide, a known GLP−1RA and GIPR antagonist, for 52 weeks, it was reported an improvement of the hs-CRP levels. However, hs-CRP changes were not linear as compared to the baseline for both groups with/without dosage escalation. In addition, the trial reported common gastrointestinal adverse effects, although with no unexpected safety signals and less frequency with lower starting dosages [204].

The results of the abovementioned experimental studies are summarized in Table 5 and Fig. 5.

2.4.3. Summary and suggestions for further studies

Overall, these studies identified adipocytes as a key site of metabolic regulation, where adipokine secretion, redox activity, and inflammatory signaling act in an integrated manner to shape adipocyte function. Activation of the GLP−1R promotes anti-inflammatory, insulin-sensitizing, and stress-reducing environment in adipocytes, according to current literature.

On the other hand, GIP signaling showed more variable effects and was often linked to pro-inflammatory responses following acute but not chronic GIPR activation in animal models. Therefore, GIPR-activation related pro-inflammatory effects should be better investigated in human-derived experimental adipocyte models to tailor GIP and GIP agonist functions.

Only GLP−1RAs and dual GIPR/GLP-RA TZP mainly showed systemic anti-inflammatory effects by ameliorating the pro-inflammatory cytokine pattern and biomarkers for tissue inflammation and endothelial dysfunction, contributing also to improve the CVD risk profile.

Future research should prioritize longitudinal and dose-dependent studies, particularly in human-derived adipocytes, to better define adipocyte-specific mechanisms and to clarify the therapeutic potential of targeting incretin pathways to modulate adipokine signaling, oxidative stress, and inflammation.

2.5. Fat browning and thermogenesis

2.5.1. Brief introduction to molecular mechanisms regulating fat browning and thermogenesis

As previously described in 2.1, according to preadipocyte lineage, human adipocytes can differentiate into white, brown, or beige adipocytes. For years, it was believed that the main WAT function was to accumulate energy by storing excessive nutrients as lipids, owing to it containing a large, single lipid droplet and few mitochondria. However, it is well known that WAT may be considered an endocrine organ since it secretes several molecules that contribute to the regulation of systemic metabolic homeostasis and significantly influence metabolic health [28].

Unlike WAT, which is the prevalent type of adipose tissue in humans, brown adipose tissue (BAT) represents a small fraction of the total adipose tissue, and it is distributed in the cervical, axillary, supraclavicular, mediastinal, paraspinal, and abdominal regions [205]. Historically, brown adipocytes were considered more metabolically active than white adipocytes owing to their peculiar histological characteristics. Indeed, they are characterized by multiple, small lipid droplets, high density of large and spherical mitochondria, and the expression of UCP−1 [206]. The latter regulates adaptive non-shivering thermogenesis by uncoupling oxidative phosphorylation from adenosine triphosphate (ATP) synthesis, dissipating energy as heat in response to both internal and external stimuli.

Table 5
Summary of experimental evidence of glucagon-like peptide−1 (GLP−1), glucose-dependent insulinotropic polypeptide (GIP), and their agonists on adipokines, oxidative stress, and inflammation.

Experimental model	Treatment	Results	Reference
In vivo			
• HFD-induced obese C57BL/6 mice	40 μg/kg semaglutide every 3 days for 4 weeks	• Improved Adipose metabolism • ↓ Tnf-α, Il−6, Mcp−1, Leptin levels in VAT (↓ inflammation)	[190]
• HFD-induced obese male C57BL/6 J mice	1.2 mg/kg TZP twice weekly for 12 weeks	• ↓ Tnf-α, Il−6, Mcp−1, Ifn-γ levels in VAT (↓ adipose inflammation) • ↓ M1 macrophage infiltration and ↑ M2 polarization in visceral fat	[191]
• Wild-type mice	24 nmol/kg [D-Ala^2]-GIP for 2 h	• ↑ serum Il−6 levels within 30 min to 2 h	[198]
• GIPR loss-of-function mice	Genetic loss of GIPR signaling	• Altered basal expression of genes involved in inflammation, thermogenesis, and lipid metabolism	[198]
• Wild-type mice	10 nmol/kg/day GIP for 4 weeks	• No effects on *Mcp−1* or *Il−6* gene expression	[200]
• Healthy and slightly obese men	2 pmol/kg/min GIP infusion for 240 min	• ↑ proinflammatory adipokine expression in SAT • ↑ serum MCP−1 levels	[199]
In vitro			
• 3T3-L1 adipocytes	10^{-10}–10^{-9} M GLP−1 for 24 h	• ↑ Visfatin secretion and gene expression in a dose- and time-dependent manner via Pka signaling • Improved redox homeostasis, ↓ ER stress and local inflammation via visfatin upregulation	[192]
• Differentiated 3T3-L1 adipocytes	2.5 × 10^{-9} M exendin−4 for 8 h	• ↑ *Adiponectin* gene expression • ↓ gene expressions of *IL−6* and *IL−18*	[193]
• hADSCs from SAT of obese subjects	10^{-9}–10^{-7} M liraglutide	• ↑ *Adiponectin* gene expression	[194]
• Brown adipose tissue cells	[10^{-7} M D-Ala^2]-GIP for 24 h	• ↑ IL−6 gene expression and secretion	[198]
• Human SGBS & monocyte-derived macrophages; murine 3T3-L1 & RAW 264.7	GIP exposure	• Inflammation dependent on adipocyte–macrophage crosstalk	[199]

Abbreviations: GIP, glucose-dependent insulinotropic polypeptide; GIPR, GIP receptor; GLP−1, glucagon-like peptide−1; GLP−1R, GLP−1 receptor; HADSCs, human adipose-derived stem cells; HFD, high-fat diet; IL, interleukin; IFN-γ, interferon-gamma; MCP, monocyte chemoattractant protein; mRNA, messenger ribonucleic acid; PKA, protein kinase A; SAT, subcutaneous adipose tissue; SGBS, Simpson–Golabi–Behmel syndrome; TNF-α, Tumor necrosis factor-alpha; TZP, tirzepatide; VAT, visceral adipose tissue.

In addition to the abovementioned adipose tissue types, a third form was also identified, namely beige AT. It has many lipid droplets, larger than those in brown adipocytes, more mitochondria than white adipocytes, and low expression levels of UCP−1. Hence, beige adipocytes may be considered an intermediate phenotype between white and brown adipocytes. Interestingly, beige adipocytes have been proposed to originate from mesodermal stem cells or from trans-differentiation of mature white adipocytes to brown adipocytes [206]. The latter is also called "fat-browning" and is finely regulated by several molecules, including transcription factors, co-regulators, non-coding RNAs, peptides, hormones, and neurotransmitters. Among these, PR domain containing 16 (PRDM−16), PPAR-γ, and PPAR-γ coactivator 1α (PGC−1α) are considered key regulators of fat browning and are needed for brown adipocyte differentiation and development.

Since increasing energy expenditure through activation of BAT may have a promising role in the treatment of metabolic diseases, huge efforts have been made in identifying molecules able to induce fat browning or improve thermogenesis. Among these, thyroid hormones [207] and β3-Ar agonists [208] were able to promote energy expenditure, inducing thermogenesis and fat browning in humans. However, these molecules were not approved for the treatment of metabolic disorders due to their associated adverse effects. The metabolic re-direction to energy expenditure rather than lipid accumulation is an intriguing long-standing research topic, and numerous studies have evaluated the role of GIP, GLP−1, and their agonists in regulating energy expenditure.

2.5.2. Evidence on modulation of fat browning and thermogenesis by GIP, GLP−1, and their agonists

Some rodent-based studies have demonstrated that treatment with GLP−1RAs may have a role in the regulation of fat browning and thermogenesis. Specifically, Han et al. reported that liraglutide (200 μg/kg/day for 8 weeks) upregulated Pgc−1a protein and *Ucp−1* transcript levels in perivascular adipose tissue from HFD-fed mice [209].

Similarly, liraglutide exposure (100 μg/kg/day for 4 weeks) induced overexpression of Ucp−1 and Prdm−16 in chow diet-fed mice, as well as in high-fat high-sucrose diet-fed mice. In a similar fashion, the cell death-inducing DNA fragmentation factor-like effector A (CIDE-A) was also over-expressed in chow diet-fed mice following liraglutide treatment [210].

In a recent study, exposure to 400 μg/kg/day liraglutide for 16 days activated BAT and induced fat browning of WAT, upregulating Ucp−1 protein expression in both tissues in HFD-fed mice. Interestingly, in the same study it was also reported that liraglutide may have an additive effect on b3-adrenergic stimulation in promoting BAT [211]. Comparable effects have been observed through the administration of 400 μg/kg/day of liraglutide for 8 weeks, inducing the expression of Ucp−1, p-Ampk-α, and p-Acc1 in epididymal and subcutaneous adipose tissues of T2DM rats [212].

Concordantly with the previous results, Martins et al. showed that liraglutide alone (200 μg/kg/day for 8 weeks), physical exercise, and their combination were able to elevate Ucp−1 protein expression in both WAT and BAT explants derived from *db/db* mice [213].

In another study, subcutaneous injection of semaglutide (40 μg/kg once every 3 days) stimulated fat browning, enhancing Ucp−1 protein expression and multiloculation in subcutaneous WAT explants from mice fed with normal or HFD [190].

Lin et al. showed that administration of exendin−4 (1.8 mg/kg) led to increased expression of *Ucp−1*, *Dio−2*, *Prdm16*, and other thermogenic-related genes in BAT, visceral and epididymal adipose tissue from HFD-fed mice. Overexpression of Ucp−1 protein levels was also confirmed by western blot assay [214].

On the other hand, an *in vivo* study reported that liraglutide failed to induce the expression of Ucp−1 or Prdm−16 in perigonadal fat of diet-induced obese mice treated with 200 mg/kg daily for 2 weeks of liraglutide [144]. Moreover, other authors showed that GLP−1RAs may modulate fat browning of WAT through an indirect mechanism. Specifically, HFD-fed mice treated with 200 μg/kg/day of liraglutide for 4 weeks showed smaller adipocytes and lipid droplets, increased expression of Ucp−1 in WAT and oxygen consumption. However, these effects were reversed after the injection of IL−6 blocking antibody, suggesting that liraglutide-induced fat browning is mediated by IL−6 [215]. Similarly, another study evaluating immortalized mouse brown pre-adipocytes cultured in brown adipogenic medium with IL−6 (500 pg/mL) and/or liraglutide (10^{-7} M) for 24 h showed that IL−6, but not liraglutide alone, induced UCP1 expression [215]. These findings

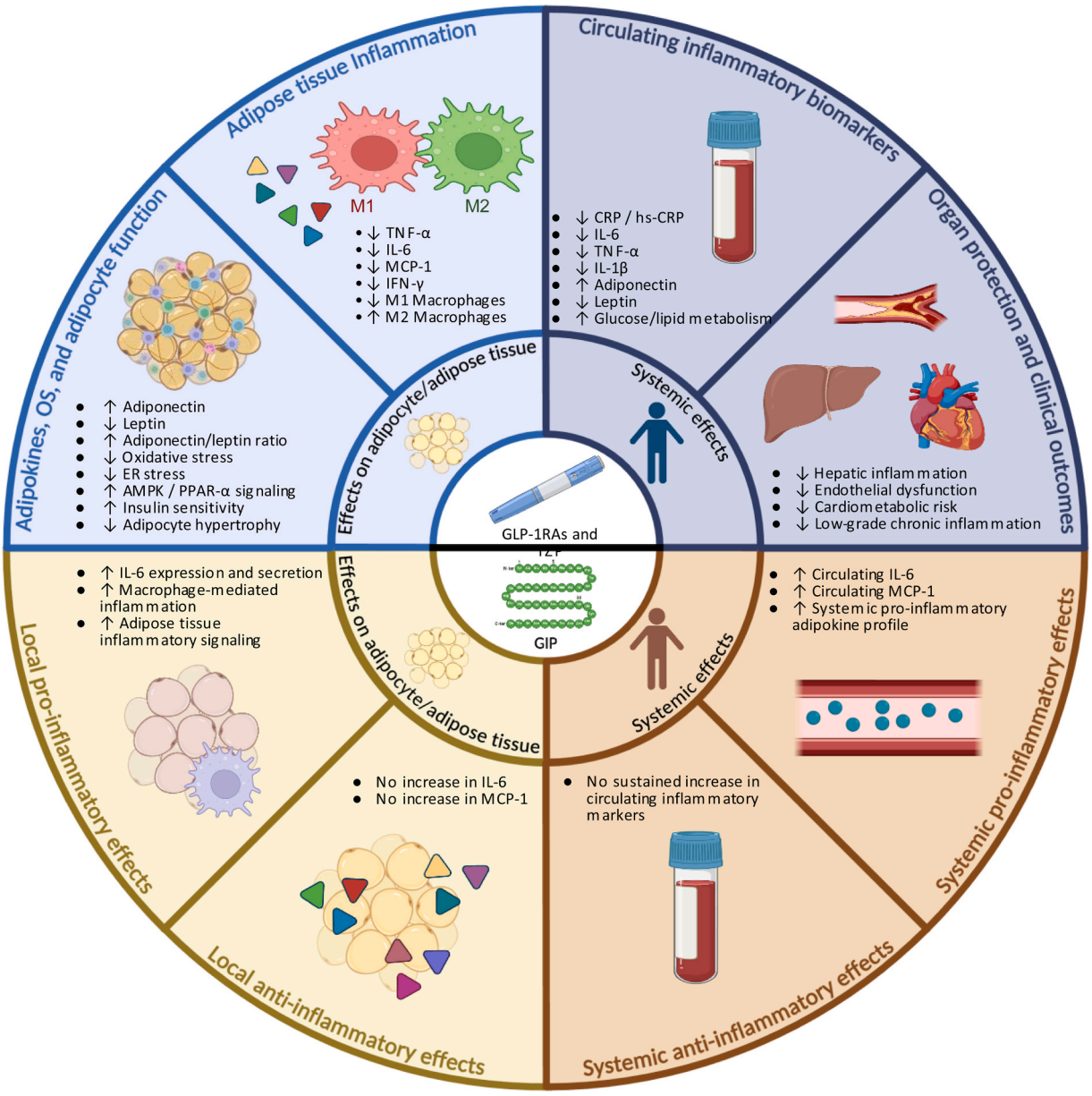

Fig. 5. Modulation of adipocyte and systemic inflammation by GIP, GLP−1, GLP−1RAs, and TZP. ***Abbreviations:*** AMPK, AMP-activated protein kinase; CRP, C-reactive protein; ER, endoplasmic reticulum; GIP, glucose-dependent insulinotropic polypeptide; GIPR, GIP receptor; GLP−1, glucagon-like peptide; GLP−1R, GLP−1 receptor; GLP−1RA, GLP−1R agonist; hs-CRP, high-sensitivity CRP, IFN-γ, interferon-γ; IL−6, interleukin−6; MCP−1, monocyte chemoattractant protein−1; OS, oxidative stress; PPARα, peroxisome proliferator activated receptor α; TNF-α,; TZP, tirzepatide.

further supported IL−6-mediated stimulatory effects of liraglutide in brown thermogenesis.

Recently, evidence from HFD-fed mice showed that exendin−4 reshaped the gut microbiota composition, enriching the abundance *Lactobacillus reuteri* in the intestine. Interestingly, the latter promoted protein expression of Ucp−1 and enhanced GLP-RA-induced fat browning when co-administered with exendin−4 (0.45 mg/kg) in mouse epididymal adipose tissue, suggesting another indirect mechanism through which GLP−1RAs may regulate fat thermogenesis [214].

Regarding *in vitro* evidence, a 2021 study evaluated the effects of GIP on fat browning in human omental adipose-derived stem cells. The results showed that treatment with 10^{-7} M of GIP increased both protein and mRNA levels of UCP−1, PGC−1α, PRDM16, whereas incubation with GIPR antagonist GIP(3−42) reversed these effects in differentiated hADSCs [216].

Li et al. reported that 3T3-L1 mouse preadipocytes treated with 10^{-7} and 10^{-6} M of liraglutide for 2 days showed a decrease of *FABP4* and *SREBP1* mRNA expression levels, whereas the thermogenic marker transcripts, including *ATGL*, *DIO−2*, *PGC−1α*, and *CIDE-A*, were upregulated. Most of these results were reversed after the incubation with 10^{-6} M sc−236, an inhibitor of cyclooxygenase 2 (COX−2), suggesting that inhibition of COX−2 may affect the GLP−1RA-mediated thermogenic activity in adipocytes. This hypothesis was also verified *in vivo* in the same paper, showing that sc−236 decreased body temperature and inhibited both mRNA and protein expression of Ucp1, Atgl, and Pgc−1α in mice exposed to cold [217].

Another study reported that liraglutide induced fat browning through regulation of miR−27b in murine adipocyte *in vitro* models. Specifically, 3T3-L1 mature adipocytes, treated with 10^{-6} M or 10^{-5} M liraglutide, showed an increase of UCP−1, PRDM−16, C/EBP-b, CIDE-A, PGC−1α transcript and protein expression levels. Following miR−27b transfection, browning-related marker mRNA and protein levels

decreased [218].

In a recent study, liraglutide was also observed to promote WAT browning both *in vivo* and *in vitro*. 3T3-L1 preadipocytes treated with increasing doses of liraglutide (10^{-8} M, 10^{-7} M, 10^{-6} M for 5 days) with normal or high-glucose medium showed a dose-dependent increase of Ucp−1 and Pgc−1α protein levels. The same was observed in the WAT from *db/db* mice treated with 400 mg/kg/day liraglutide for 8 weeks [219].

Conversely with these results, 10^{-7} and 10^{-8} M liraglutide treatment failed to promote the expression of lipolysis-related genes, including *Atgl*, *Hsl*, and *hepatic lipase* (*Lip*) *C*, and protein expression of Ucp−1, Hsl, and Atgl in mature 3T3-L1 adipocytes. The authors also reported that liraglutide may promote indirectly lipolytic activity and browning by inducing hepatic, pancreatic and VAT Fndc5 expression in HFD-induced obese mice [220].

A recent study explored the signaling pathways involved in the GLP−1RA-induced thermogenesis *in vitro*. Particularly, 24-hour 10^{-9} M, 2×10^{-8} M, or 10^{-7} M exendin−4 exposure induced upregulation of Sirt−1, Ppar-α, Pgc−1α and Ucp−1 protein expression levels, and activation of Ampk/Acc pathway and Atgl in 3T3-L1 adipocytes. These effects were reversed after incubation with Sirt−1 inhibitor, suggesting that exendin−4 may affect mitochondrial activity in adipocyte by regulating Sirt−1 expression [221].

On the other hand, an *in vitro* study found GLP−1R expression in both human undifferentiated and differentiated SGBS adipocytes. Additionally, long-term treatment with increasing doses of liraglutide (10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M for 15 days) promoted protein expression of UCP−1 and upregulated mRNA levels of genes involved in fat browning such as PPAR-γ, PGC−1α, PRDM16, CIDE-A, and transmembrane protein 26 (TMEM26) in human differentiated SGBS adipocytes [222].

Interestingly, dual GIPR/GLP−1R agonist TZP was found to promote BAT thermogenesis and WAT fat browning by upregulating protein levels of thermogenic-related genes in obese mice, following both acute (2×10^{-5} M, single injection) and chronic (2×10^{-6} M for 10 days) intracerebroventricular administration [223]. These findings support TZP-driven metabolic shifting both in brown and white AT. The results of the abovementioned studies are summarized in Table 6.

2.5.3. Summary and suggestions for further studies

Only one study evaluated the involvement of GIP in fat browning and thermogenesis in human adipose tissue [216]. For GLP−1 and its agonists, several *in vivo* and *in vitro* studies demonstrated that these molecules may have a role in the regulation of thermogenesis-related gene expression at different concentrations and time of exposure [190,209–213,217,218,219,221,222]. However, other studies reported conflicting results [144,220] or the involvement of indirect mechanisms mediating the effects of GLP−1RAs in mice [214,215].

Moreover, most of the above-mentioned results derive from animal cell cultures [217–221] and few papers [216,222] have evaluated the regulation of thermogenesis and fat browning by GIP, GLP−1, and their agonists in human adipocytes.

Given that these hormones may elicit species-specific physiological responses, these results may lead to misleading interpretations and should be carefully evaluated. Accordingly, they should be confirmed in robust human-derived cellular models.

Regarding dual GIPR/GLP−1RAs, only one study showed that both acute and chronic central infusion of TZP may improve energy expenditure in mice [223]. Of note, to the best of our knowledge, no study has evaluated the direct effects of dual GIPR/GLP−1RAs on fat browning or thermogenesis in human adipocytes. Accordingly, this is an intriguing topic that should be investigated in the next future.

3. Discussion and future perspectives

Clinical evidence demonstrated that incretin-based drugs are effective and safe therapeutic approaches in weight loss. GLP−1RAs and dual GIPR/GLP−1RA-related body weight reduction may be attributed, at least in part, to incretin modulation of food intake. Currently, their central effects on molecular mechanisms regulating feeding behavior were partially investigated and not fully understood. It is known that food intake is mainly regulated centrally by the integration of multiple signals into the brain, specifically at the hypothalamic level [224]. Particularly, several hormones including ghrelin, insulin, leptin, GLP−1, and peptide tyrosine (PYY), reach the arcuate nucleus in the hypothalamus and interact with orexigenic neuropeptide Y (NPY) and agouti-related peptide (AgRP) neurons and anorexigenic pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) neurons [225,226]. These neuron subsets project to paraventricular nucleus and lateral hypothalamic area, finely regulating hunger and satiety [225]. Within this context, ghrelin can stimulate orexigenic NPY/AgRP neurons [225,226], influencing feeding behavior via central action and promoting fat storage peripherally, especially with aging [227]. On the other hand, insulin, leptin, and PYY inhibit these neurons, while leptin and GLP−1 are able to directly activate anorexigenic POMC/CART neurons [225,226]. Accordingly, feeding behavior is orchestrated by highly redundant and complex mechanisms, involving several hormones and neural circuits. After weight loss, counter-regulatory response, modulating food intake-related hormones and body energy expenditure, is triggered to maintain body weight, which may drive weight regain. In this context, a thorough knowledge of incretin-based therapy effects on central weight maintenance and regain mechanisms is required to improve long-term weight loss.

Beyond central food intake regulation, significant knowledge gaps remain to be clarified about the human adipose tissue-specific effects induced by GLP−1RAs and dual GIPR/GLP−1RAs.

To better investigate molecular mechanisms behind human adipose tissue functions, *in vitro* studies focused on human-derived cell models may have a role in elucidating the mechanistic basis of clinical observations. This reverse translational approach, through the identification of novel molecular mechanisms, may further stimulate and refine both clinical and pharmacological research.

In this review, we summarized the main results from preclinical studies on incretin-based treatment in human- and animal-derived adipocytes. Overall, aforementioned studies reported that GIP, GLP−1 and their agonists may regulate different aspects of adipocyte function. However, some constraints should be taken into account. Most of the studies evaluated the effects of these molecules in rodent cellular and animal models which could exhibit limited translational potential, due to significant metabolic differences compared to humans. On the other hand, we found very few studies on human-derived cellular models. Furthermore, *in vitro* evidence gathered from human-derived cell models has some limitations that should be considered. Accordingly, SGBS cellular models may be influenced by the genetic background of the syndrome, whereas *ex vivo* human cells may be affected by the wide spectrum of phenotypical characteristics of selected subjects. Therefore, more robust human-based cellular models which better recapitulate human adipocyte pathophysiology are needed to improve translational power of *in vitro* evidence to real-world applications.

Moreover, in the context of obesity-related metabolic disorders, human dysfunctional adipocytes secrete pro-inflammatory factors, altering redox homeostasis and inducing local and systemic inflammation, that in turn worsen adipose tissue dysfunction and promote a vicious cycle fueling metabolic derangements. This pro-inflammatory environment may also involve other cell actors, including local stromal, immune, and endothelial cells. Therefore, it has to be mentioned that single-adipocyte cultures, whether using functional or dysfunctional adipocytes, are unable to comprehensively replicate both local and systemic interactions between adipose and other tissues or organs.

Notably, three-dimensional (3D) cell models more consistently recapitulate the multicellular complexity and physiology of tissues.

Table 6
Summary of the experimental evidence of glucagon-like peptide−1 (GLP−1), glucose-dependent insulinotropic polypeptide (GIP), and their agonists on fat browning and thermogenesis.

Experimental model	Treatment	Results	Reference
In vivo			
• HFD mice	200 μg/kg/day liraglutide for 8 weeks	• ↑ expression of Pgc−1α protein and *Ucp−1* transcript in PVAT	[209]
• Mice fed with chow or HFHS diet	100 μg/kg/day liraglutide for 4 weeks	• ↑ protein expression of Ucp−1 and Prdm−16 in perigonadal adipose explants from both chow and HFHS diet mice • ↑ protein expression of Cide-A in perigonadal adipose explants from chow diet mice	[210]
• HFD mice	400 μg/kg/day liraglutide for 16 days	• ↑ protein expression of Ucp−1 in BAT and WAT	[211]
• T2DM mice	400 μg/kg/day liraglutide for 8 weeks	• ↑ protein expression of Ucp−1, p-Ampk-α, and p-Acc1 in epididymal and subcutaneous adipose explants	[212]
• *db/db* mice	200 μg/kg/day liraglutide for 8 weeks	• ↑ protein expression of Ucp−1 in both WAT e BAT explants	[213]
• Mice fed with chow or HFD	40 μg/kg liraglutide once every 3 days	• ↑ protein expression of Ucp−1 in WAT • Multiloculation in subcutaneous WAT	[190]
• HFD mice	1.8 mg/kg exendin−4	• ↑ gene expression of Ucp−1, Dio−2, Prdm16, and other thermogenic-related genes in BAT, visceral and epididymal adipose tissue • ↑ protein expression of Ucp−1	[214]
• Obese mice	200 mg/kg/day liraglutide for 2 weeks	• No effects on expression of Ucp−1 or Prdm−16 in perigonadal fat • ↓ adipocyte and lipid droplet sizes	[144]
• HFD mice	200 μg/kg/day liraglutide for 4 weeks	• ↑ protein expression of Ucp−1 in WAT and oxygen consumption via IL−6 signaling	[215]
• HFD mice	Intracerebroventricular injection of 2×10^{-5} M TZP	• ↑ gene and protein expressions of Ucp−1, Pgc−1α, and Dio−2 in BAT • ↑ protein expression of Ucp−1, Pgc−1α, and Dio−2 in WAT • ↑ gene and protein expressions of Ucp−1, Pgc−1α, and Dio−2 in WAT and BAT	[223]
• *db/db* mice	400 μg/kg/day liraglutide for 8 weeks	• ↑ protein expression of Ucp−1 and Pgc−1α in WAT	[219]
In vitro			
• Human omental adipose-derived stem cells	10^{-7} M GIP	• ↑ gene and protein expressions of UCP−1, PGC−1α, PRDM16	[216]
• 3T3-L1 mature adipocytes	10^{-7} M and 10^{-6} M liraglutide for 2 days	• ↑ gene expression of *Atgl*, *Dio−2*, *Pgc−1α*, and *Cide-A*	[217]
• 3T3-L1 pre-adipocytes	10^{-8} M, 10^{-7} M, and 10^{-6} M liraglutide for 5 days	• ↑ protein expression of Ucp−1 and Pgc−1α in a dose-dependent manner	[219]
• 3T3-L1 mature adipocytes	10^{-8} M and 10^{-7} M liraglutide	• No effects on *Atgl*, *Hsl*, and *hepatic Lip-C*, and protein expression of Ucp−1, Hsl, and Atgl	[220]
• Immortalized mouse pre-adipocytes	10^{-7} M liraglutide for 24 h	• No effects on Ucp−1 expression	[215]
• 3T3-L1 adipocytes	10^{-9} M, 2×10^{-8} M, and 10^{-7} M exendin−4 for 24 h	• ↑ protein expression of Sirt−1, Ppar-α, Pgc−1α, and Ucp−1 • Activation of Ampk/Acc pathway and Atgl	[221]
• SGBS adipocytes	10^{-6} M, 10^{-7} M, 10^{-8} M, and 10^{-9} M liraglutide for 15 days	• ↑ protein expression of UCP−1 and gene expressions of *PPAR-γ*, *PGC−1α*, *PRDM16*, *CIDE-A*, and *TMEM26*	[222]

Abbreviations: ACC, acetyl-CoA carboxylase; AMPK, adenosine monophosphate-activated protein kinase; ATGL, adipose triglyceride lipase; BAT, brown adipose tissue; C/EBP-β, CAAT enhancer binding protein-β; CIDE-A, cell death-inducing DNA fragmentation factor-like effector-A; DIO−2, iodothyronine deiodinase−2; GIP, glucose-dependent insulinotropic polypeptide; HFD, high-fat diet; HFHS, high-fat high-sucrose; HSL, hormone sensitive lipase; LIP-C, hepatic lipase-C; PGC−1α, PPAR-γ coactivator−1α; PPAR-α, peroxisome proliferator-activated receptor-α; PRDM16, PR domain containing−16; PVAT, perivascular adipose tissue; SGBS, Simpson-Golabi-Behmel syndrome; SIRT−1, Sirtuin−1; TMEM26, transmembrane protein 26; T2DM, type 2 diabetes mellitus; TZP, tirzepatide; WAT, white adipose tissue; UCP−1, uncoupling protein−1.

Owing to their characteristics, adipose tissue organoids have shown the potential to accurately mimic the heterogeneity and complexity of human adipose tissue pathophysiology. However, these models lack structural complexity, result in highly variable sizes, and have limited potential for large-scale and standardized production [228].

An intriguing topic that deserves further investigation in the next future is the evaluation of incretin-based therapy effects on 3D organoids including the main adipose tissue-derived cellular components to elucidate underlying molecular mechanisms orchestrating the crosstalk between adipocytes and other cellular types within adipose tissue dysfunction.

On the other hand, as mentioned above, GIPRs and GLP−1Rs are widely expressed in several human tissues. Accordingly, metabolic effects of GLP−1R and dual GIPR/GLP−1RAs may be influenced by complex interactions of multiple signaling pathways occurring across different organs and tissues. All these aspects should be considered when translating experimental evidence into clinical practice.

To bridge the gap between preclinical and clinical evidence, multi-omics data integration and development of advanced bioinformatics tools may broaden our understanding of systemic mechanisms affected by obesity-related metabolic dysfunction. Indeed, multi-omics data integration combining epigenomics, genomics, transcriptomics, proteomics, and metabolomics has shown potential to provide a thorough and holistic view of pathophysiological processes [229–231].

Furthermore, integration of multiple data layers within machine learning-based algorithms, deep learning and artificial intelligence tools may be useful for development of risk prediction models for metabolic disorder-related comorbidities. The combination of multi-omics data analysis integration with such bioinformatic tools may also allow identification of novel biological targets of metabolic dysfunction [232], as well as enable the tailoring of more appropriate and personalized therapeutic strategies based on individual molecular profiles [233].

In addition to these promising predictive tools, several new molecules that move beyond body weight loss and involve novel mechanisms, including targeting other gut-related hormones, metabolic enzymes, or lean body mass, are currently under development. Indeed, an emerging issue of current pharmacological therapy of obesity is the reduction of lean body mass [234,235], raising concerns regarding the safety profile of these drugs in patients with sarcopenic obesity. These data highlight the need for novel strategies to prevent lean body mass loss in patients at higher risk for sarcopenia and its sequelae.

In this regard, bimagrumab is a novel monoclonal antibody which binds to activin A receptor (ActR) type IIA/IIB, inhibiting its effects in skeletal muscle cells. In physiological conditions, TGF-β family ligands, including activin, myostatin, and TGF-β, downregulate signaling pathways involved in muscle growth and differentiation, leading to muscle atrophy by binding ActR-IIA/IIB [236]. In a recent phase 2 randomized clinical trial, T2DM patients treated with bimagrumab (10 mg/kg up to 1200 mg) every 4 weeks for a total of 48 weeks showed a significant reduction of fat mass (average −20.5%, −7.5 kg [80% confidence interval (CI), −8.3 to −6.6 Kg]) and a slight increase of lean mass (average +3.6%, 1.70 kg [80% CI, 1.1–2.3 kg]) in patients with obesity. In addition, a decrease in hepatic fat content and abdominal visceral fat were also reported in the same study. On the other hand, the most frequent adverse effects were diarrhea (41%), muscle spasms (41%), upper respiratory tract infection (16%), increased lipase level (11%), and nausea (11%), while therapy discontinuation occurred in 14% of patients [237].

BMS−963272 is a potent inhibitor of monoacylglycerol-O-acyl transferase (MGAT) −2 resulting in increased FFA accumulation in enterocytes, as well as in hepatocytes [238]. Interestingly, BMS−963272 has been shown to increase GLP−1 and PYY secretion by enterocytes as well as their circulating levels in mice and in humans [239], likely by promoting intracellular FFA accumulation. In the same study, administration of BMS−963272 (300 mg/8 h/14 days) was associated with –1.7 Kg mean weight loss in patients with obesity. Furthermore, authors reported that BMS−963272 was well tolerated and no treatment discontinuation due to adverse effects [239].

Among next-generation pharmacological treatments that are currently in phase III trials, amylin analogs showed promising results in obesity management. Amylin belongs to the calcitonin peptide family, and it is produced and co-released with insulin from pancreatic β-cells after meal. By binding its own receptor, amylin can modulate several signaling pathways involved in food intake, energy expenditure, and food reward [240]. Recent randomized clinical trials showed that long-acting amylin analogue (cagrilintide, 4.5 mg for 26 weeks) alone or in combination with semaglutide (cagrisema; cagrilintide 2.4 mg/semaglutide 2.4 mg, for 68 weeks) were able to promote significant weight loss (up to –10.8% [241] and –20.4% [242]) in patients with obesity. Gastrointestinal adverse effects, including nausea, constipation, and diarrhea, and administration-site reactions were the most frequent adverse events and treatment discontinuation rates were similar to those reported for GLP−1R agonists [241].

Regarding other innovative incretin-based molecules, orforglipron showed great weight loss efficacy in patients with obesity. Orforglipron, recently approved by FDA, is an oral small-molecule GLP−1R agonist that is able to induce downstream signal transduction with negligible β-arrestin recruitment [243]. Owing to orforglipron has non-peptide molecular structure, it is not degraded by proteolytic enzymes which are abundant in the gastrointestinal tract, overcoming the subcutaneous injection [244].

In a recent phase III randomized clinical trial, this molecule significantly decreased mean body weight (6 mg: −7.5%, [95% CI, −8.2% to −6.8%]; 12 mg: −8.4% [95% CI, −9.1% to −7.7%]; 36 mg: −11.2% [95% CI, −12.0 to −10.4] compared to placebo (−2.1% [95% CI, −2.8% to −1.4%]) in patients with obesity (mean BMI 37 Kg/m^2) after 72 weeks [245]. Similarly to other GLP−1R agonists, the most common adverse effects were mild to moderate gastrointestinal disorders [245].

According to this preliminary data this molecule has the potential to mitigate the limitations of current oral GLP−1RA therapy, offering an alternative approach to patients who prefer oral to injective drugs.

However, clinical trials also showed higher adverse event-related therapy discontinuation (5.3–10.3%) [245] compared to injective formulation of semaglutide (5.9%) [16] and tirzepatide (4.3–6.2%) [17] in patients with obesity. Similar data were reported in patients with T2DM by ACHIEVE−3 clinical trial (9–10%) [246].

Other interesting targets that may improve metabolic effects of incretin-based therapy are GCG and its own receptor. GCG is secreted by pancreatic α-cells promoting hepatic glycogenolysis and lipolysis. Evidence of GCG-mediated lipolytic effects in adipocytes was observed in animal-derived adipocytes, including rats [247–249] and birds [250]. Accordingly, GCG can induce the activation of AC by binding its own receptor, leading to increased intracellular cAMP. cAMP in turn activates PKA which phosphorylates and activates HSL and perilipins, resulting in triglyceride breakdown into diacylglycerols and then monoacylglycerols [251]. Furthermore, GCG-mediated activation of monoacylglycerol lipase drives FFA and glycerol releases [251]. On the other hand, GCG downstream signaling results in ACC inactivation which inhibits lipogenesis [252]. However, contrasting results have emerged from different studies based on rat adipocytes showing that GCG did not regulate, specifically, WAT lipolysis [253]. On the other hand, the effects of GCG on lipid metabolism in human adipocytes remain debated. Some authors showed that GCG stimulated lipolysis in human adipocytes [254,255], while in others it did not affect lipid metabolism [256,257]. Recently, a study collected adipose tissue biopsies from both patients with T2DM and controls showing that GCGR was differently expressed in SAT and VAT and varied between individuals. GCG slightly stimulated lipolysis in human adipocyte at supra-physiological concentrations [258]. However, *in vivo* and *in vitro* studies showed that insulin reverted GCG-mediated lipolysis [258,259], suggesting that GCG may promote lipolysis only during fasting in adipose tissue. Currently, GCG-mediated lipolytic effects in human-derived adipocytes remain

poorly investigated.

Furthermore, GCG is also involved in other metabolic processes, including food intake, energy expenditure, and protein metabolism, suggesting that GCGR may be an interesting target to shape body weight and composition. To this end, dual GLP−1R/GCGR and triple GLP−1R/GIPR/GCGR agonists are currently under development for the treatment of obesity and T2DM. Among these molecules, according to preliminary preclinical data, triple agonist was superior to any existing dual agonists in weight loss and regulation of glycemic metabolism in mice [260].

Retatrutide is a 39 amino acid single peptide engineered from a GIP peptide backbone with triple GLP−1R/GIPR/GCGR agonist activity [261]. Clinical evidence reported that treatment with 1–12 mg retatrutide for 48 weeks led to a reduction in mean body weight up to 24.2% in patients with mean BMI 37.3 Kg/m^2. The most frequent adverse events were gastrointestinal disorders, and treatment discontinuation occurred in 6–16% of patients [262]. Similar results were reported by Coskun et al., showing that the proportion of lean mass loss to weight loss was similar to other obesity treatments [263]. Interestingly, in another clinical study, treatment with retatrutide showed a dose-dependent reduction in fat liver content in a subgroup of patients [264], suggesting a potential therapeutic role in patients with MASLD.

Regarding experimental evidence, a recent study reported that retatrutide increased energy expenditure via glucagon signaling in mice. Moreover, the same study showed that retatrutide promoted lipolysis in differentiated human primary adipocytes [261]. However, whether lipolysis was mediated by activation of GIPR and/or GCGR-related signaling pathways was not evaluated. Further *in vitro* studies elucidating the GCGR expression in human adipocytes as well as if retatrutide may directly affect lipid metabolism through GCGR signaling in these cells are relevant topics requiring further investigations.

Although incretin-based therapy has reshaped clinical management of patients with obesity, this pharmacological approach has faced several constraints, including tolerance development or suboptimal response to therapy, gastrointestinal adverse effects, and weight regain upon discontinuation.

STEP−5 and SURMOUNT−1 clinical trials respectively reported that 77.1% [16] and 90.9% [17] of patients lost 5% or more of their body weight with the highest dose of semaglutide and tirzepatide. According to these clinical trials, monomolecular multi-agonists promoted weight reduction in a wider population, suggesting that a multi-target approach may enhance mono-agonist efficacy. Notably, almost 10% of patients treated with tirzepatide (15 mg) showed less than 5% body weight reduction [17]. These results underline the necessity of a broader knowledge on molecular mechanisms regulating the individual response to incretin-based therapy.

Regarding the safety profile, incretin-based mono- and multi-agonists are generally well tolerated in most patients. However, incretin-based pharmacotherapy-induced gastrointestinal adverse effects have been frequently reported and may lead to treatment discontinuation in some cases, raising concerns about their tolerability. These adverse events have often of mild or moderate intensity occur early and are self-limited over time. In limited instances, they may become severe or persist too long to be well tolerated. Slow titration (dosage escalation in more steps) may be a solution for patients prone to develop gastrointestinal disorders. Moreover, symptomatic treatment may also be considered to alleviate gastrointestinal symptoms in some patients.

In addition, patients with obesity may also discontinue incretin-based pharmacotherapy for its relatively high cost. Owing to obesity is a chronic relapsing disease that requires long-term treatment, anti-obesity drugs suspension will consistently contribute to weight regain, unless body weight maintenance is addressed with lifestyle interventions. In this regard, future availability of generic drugs should improve access and economic sustainability of anti-obesity long-term treatments.

According to the results of aforementioned studies, the therapeutic landscape for obesity management will rapidly expand in the close future, offering a wide spectrum of molecules with different pharmacological properties and mechanisms of action that may overcome some limitations of current obesity pharmacotherapies. However, several concerns, especially those related to the heterogeneity of clinical responses to incretin-based therapy, require further elucidation.

4. Conclusion

The role of incretin-based therapy in the management of obesity is supported by a growing body of evidence. Such pharmacological therapy should be considered in selected patients in the context of a multidimensional approach, promoting a healthy lifestyle. Incretin-based therapy may promote weight loss and mitigate obesity-related comorbidities by acting at central and peripheral sites, including adipose tissue and adipocytes. Indeed, current experimental evidence supports the role of GLP−1R and dual GIPR/GLP−1RAs in reshaping adipocyte metabolic dysfunction. Future research will address existing knowledge gaps in this field by taking advantage of novel experimental models, such as dysfunctional human-derived adipocyte systems.

In addition, integration of these multidimensional approaches, involving human-derived multi-cellular models, multi-omics data integration, machine learning, and artificial intelligence tools, with the introduction of these well-promising drugs could play a crucial role in redirecting clinical management of metabolic disorders toward personalized and precision-based medicine.

CRediT authorship contribution statement

Aldo E. Calogero: Investigation. **Sandro La Vignera:** Validation. **Rosita A. Condorelli:** Methodology. **Rossella Cannarella:** Investigation. **Claudia Giglione:** Writing – review & editing, Writing – original draft, Supervision, Methodology. **Reham Hattab:** Writing – original draft, Methodology. **Hygerta Berisha:** Writing – original draft, Methodology. **Paolo Magni:** Writing – review & editing, Project administration, Formal analysis, Conceptualization. **Roberto Curto:** Writing – review & editing, Writing – original draft, Methodology, Investigation.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors declare that they have not used generative AI and AI-assisted technologies during the manuscript preparation process.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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