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Review

Knocking on GDF15's door for the treatment of type 2 diabetes mellitus

David Aguilar-Recarte,[1,2,3,4,8] Emma Barroso,[1,2,3,4,8] Xavier Palomer,[1,2,3,4] Walter Wahli,[5,6,7] and Manuel Vázquez-Carrera[1,2,3,4,*]

Although a large number of drugs are available for the treatment of type 2 diabetes mellitus (T2DM), many patients do not achieve adequate disease control despite adhering to medication. Recent findings indicate that the pharmacological modulation of the stress-induced cytokine growth differentiation factor 15 (GDF15) shows promise for the treatment of T2DM. GDF15 suppresses appetite and reduces inflammation, increases thermogenesis and lipid catabolism, sustains AMP-activated protein kinase (AMPK) activity, and ameliorates insulin resistance and hepatic steatosis. In addition, circulating GDF15 levels are elevated in response to several antidiabetic drugs, including metformin, with GDF15 mediating some of their effects. Here, we review the mechanistic insights into the beneficial effects of recently explored therapeutic approaches that target GDF15 for the treatment of T2DM.

Highlights

Despite the high number of drugs currently available for the management of type 2 diabetes mellitus (T2DM), many patients do not achieve adequate disease control, emphasizing the need for new drugs.

Targeting growth differentiation factor 15 (GDF15), a cytokine induced by the integrated stress response (ISR), has emerged as a therapeutic option for obesity and T2DM.

GDF15 reduces food intake and body weight through its central receptor glial cell line-derived neurotrophic factor (GDNF)-like alpha-1 (GFRAL), but its peripheral effects through an unknown receptor also contribute to ameliorating insulin resistance.

Encouraging findings in animal models indicate that GDF15-based therapies are promising for the treatment of obesity and T2DM, but clinical studies in humans are needed to confirm this.

T2DM: a rising tide

T2DM is a complex metabolic disorder chiefly characterized by hyperglycemia, with an incidence projected to increase by more than 10% by 2040 [1]. The increase in the incidence of T2DM is especially dramatic in adolescents and young adults, since young-onset T2DM has a more aggressive disease phenotype, leading to the premature development of complications [2]. Insulin resistance (IR), the hallmark of T2DM, precedes and predicts the development of T2DM and is defined as a defect in the capacity of insulin to drive glucose to its target tissues [3]. Frequently, one of the early alterations observed in subjects prone to IR/T2DM is the accumulation of intra-abdominal adipose tissue. In fact, the epidemiological relationship between obesity and IR is well established [4]. The hepatic manifestation of IR is **nonalcoholic fatty liver disease (NAFLD)** (see Glossary), which is increasingly recognized as the most common chronic liver disease affecting 25% of the global population [5]. NAFLD predicts the development of T2DM and vice versa, with each condition possibly serving as a progression factor for the other [6].

Although a large number of drugs are currently available for the management of T2DM, many patients do not achieve adequate disease control despite adhering to medication [7,8], emphasizing the need for new drugs. Recent findings indicate that the pharmacological modulation of the stress-induced **cytokine** GDF15 shows promise as a potential therapeutic strategy for the treatment of T2DM. This review highlights the current understanding of the actions of GDF15 on T2DM and of how pharmacological therapies based on GDF15 may become future treatments for this disease.

GDF15 is a stress-induced cytokine

The development of T2DM occurs as a result of the combination of IR and a defective insulin secretion by β cells. Obese subjects at risk of developing T2DM may display an initial state of IR. Under these conditions, β cells increase insulin secretion to compensate for the reduced

[1]Department of Pharmacology, Toxicology and Therapeutic Chemistry, Faculty of Pharmacy and Food Sciences, University of Barcelona, Barcelona, Spain
[2]Institute of Biomedicine of the University of Barcelona (IBUB), Barcelona, Spain
[3]Pediatric Research Institute-Hospital Sant Joan de Déu, Barcelona, Spain
[4]CIBER de Diabetes y Enfermedades Metabólicas Asociadas, Instituto de Salud Carlos III, Avinguda Joan XXII 27-31, E-08028 Barcelona, Spain
[5]Center for Integrative Genomics, University of Lausanne, CH-1015 Lausanne, Switzerland
[6]Lee Kong Chian School of Medicine, Nanyang Technological University Singapore, Singapore 308232
[7]ToxAlim (Research Center in Food Toxicology), INRAE, UMR1331, 31300 Toulouse Cedex, France

action of insulin, maintaining glucose tolerance. However, over time, β cell insulin secretion declines, leading to insufficient insulin levels that, in a context of IR, results in **glucolipotoxicity** and obesity-induced inflammation, ultimately causing overt hyperglycemia that eventually leads to the diagnosis of T2DM [9].

In recent years, mounting evidence for a close link between a state of **chronic low-level inflammation** and the development of IR/T2DM has emerged. In fact, several molecules released by adipocytes – such as inflammatory cytokines and free fatty acids (FFAs) – trigger inflammatory pathways in different tissues. Skeletal muscle, the liver, and the heart, which undergo marked molecular remodeling in response to a lipid overload, secrete proteins that can drive inflammation, similar to adipose tissue. To limit these deleterious processes, cells secrete stress-responsive cytokines such as GDF15, which activate counteracting mechanisms that inhibit inflammation, IR, and NAFLD [10]. The pharmacological overactivation of these mechanisms offers new therapeutic strategies to prevent inflammation and IR/T2DM.

In healthy human subjects, circulating serum GDF15 levels range from 200 to 1000 pg/ml (50–100 pg/ml in mice), but these levels are remarkably elevated under conditions of cellular stress such as in several diseases (especially in cancer, cardiovascular disease, obesity, mitochondrial diseases, and aging) [10–14] (Box 1). In fact, the presence of increased levels of this cytokine in subjects suffering from many of these diseases has led to GDF15 being considered as a biomarker of these pathologies, being associated with overall mortality [15].

GFRAL and the central role of GDF15 in regulating food intake

Consistent with the classification of GDF15 as a divergent member of the **transforming growth factor β (TGF-β)** superfamily, initial studies reported that the effects of GDF15 were mediated by the TGF-β receptor types I/II. However, the lack of binding of GDF15 to these receptors and the presence of TGF-β contamination in the first purified GDF15 batches from mammalian cell cultures, which affected the interpretation of some *in vitro* studies, led to a search for other receptors [16]. In 2017, four independent research groups identified glial cell-derived neurotrophic factor family receptor α-like (GFRAL) as the cognate receptor for GDF15 [17–20]. Interestingly, the expression of this receptor is restricted to the neurons of the area postrema and solitary tract nucleus, which are brain regions involved in appetite and weight regulation. The binding of GDF15 to GFRAL leads to the formation of a complex with the rearranged during transfection (RET) coreceptor, the tyrosine kinase activity of which leads to the activation of the extra cellular signal-regulated kinase (ERK), protein kinase B (AKT), and phospholipase Cγ (PLCγ) pathways. The activation of these pathways by the GDF15–GFRAL axis results in an anorectic effect that causes a reduction in body weight [17–20]. This role of GDF15 in appetite regulation is consistent with previous studies showing that the remarkable increases in GDF15 levels caused by tumor implantation in mice lead to weight loss [21]. In addition, transgenic mice overexpressing *Gdf15* show a reduction in food intake and a lean phenotype, and are more resistant to obesity, metabolic inflammation, and glucose intolerance [21–25]. Likewise, *Gdf15* and *Gfral* knockout mice fed a high-fat diet (HFD) display a slight increase in fat depots and body weight when compared with wild-type mice [19,26]. In line with these findings, the administration of either a single dose or chronic doses of recombinant GDF15 (rGDF15) reduced food intake and body weight in mice, but these effects were absent in *Gfral* knockout mice [17,19,20]. The suppressive effect of GDF15 on food intake seems to be mediated by nausea, emesis, and taste aversion through GFRAL. In fact, it has been reported that the administration of rGDF15 induces signs of nausea and emesis in musk shrews, which possess the vomit reflex (in contrast to rodents) [27]. These data suggest that GDF15 induces nausea and emesis, which precede anorexia, and are involved in the weight loss elicited by this cytokine [28]. Interestingly, the discovery of this emetic effect of

[8]These authors contributed equally to this work.

*Correspondence:
mvazquezcarrera@ub.edu
(M. Vázquez-Carrera).

GDF15 has led to the development of antibodies against GDF15 for the treatment of cancer cachexia or to reduce emesis in chemotherapy [27,29,30]. The emetic effect of GDF15 might be an obstacle for the development of anti-obesity and antidiabetic therapies based on this cytokine, and pharmacological strategies based on GDF15 should try to attenuate this effect. In fact, this side effect of GDF15 seems to be dose-dependent, since the lowest efficacious dose of GDF15 did not cause emesis in musk shrews [28], suggesting that it can be reduced by using dose titration studies. Moreover, we should bear in mind that long-acting glucagon-like peptide 1 receptor (GLP-1R) agonists used in the treatment of obesity and T2DM cause mild to moderate nausea as a side effect, although this is attenuated with treatment progression [31]. In addition to nausea and emesis, GDF15 might reduce obesity by inducing a conditioned taste aversion [11,27], affecting food preferences that reduce fat consumption [20,32], and by delaying gastric emptying through a vagal-mediated mechanism [32].

Since obese subjects and obese mice display increased levels of circulating GDF15, it has been suggested that obesity induces a state of resistance to the anorexigenic effects of GDF15 [11]. This resistance to GDF15 in obesity might be mediated by membrane-bound matrix metalloproteinase 14 (MT1-MMP/MMP14), which functions as an endogenous negative regulator of GFRAL. In fact, activation of MT1-MMP in obese rodents proteolytically inactivates GFRAL, thereby suppressing GDF15–GFRAL signaling and eventually reducing the anorectic effects of GDF15 [33]. These observations identify MT1-MMP as a promising pharmacological target for the treatment of obesity and associated metabolic diseases such as T2DM.

Collectively, these findings indicate that GDF15 reduces food intake and body weight through several central mechanisms, but it remains to be confirmed whether these actions of GDF15 – some of which have so far been observed only in animal models – fully operate in humans.

Peripheral effects of GDF15

Although many of the beneficial effects of GDF15 on glucose homeostasis are mediated by the reduction in food intake and body weight [18,20], several lines of evidence indicate that GDF15 may also have peripheral effects. Indeed, GDF15 increases thermogenesis, lipid catabolism, and mitochondrial oxidative phosphorylation independently of changes in food intake [34], suggesting that GDF15 might also exert its effects through receptors other than GFRAL and independently of appetite reduction. It has been proposed that the anorectic effect of GDF15 involving GFRAL requires a threshold of circulating GDF15 levels that is ≥400 pg/ml in mice, with lower values not affecting appetite [35]. Consistent with this, a recent study showed that the anorectic effect via GFRAL of exogenously administered GDF15 occurred only at high pharmacological doses, with physiological increases (two- to threefold) of endogenous GDF15 levels (basal values of ~50 pg/ml in mice) not suppressing food intake [36]. Indeed, a GDF15 dose of 20 μg/kg that resulted in serum levels of ~1500 pg/ml reduced food intake, whereas a lower dose of GDF15 of 2 μg/kg had no effect on food intake at any of the time points studied [27]. Supporting a peripheral effect of GDF15, we recently reported that GDF15 activates AMPK in cultured myotubes and isolated skeletal muscle that do not express GFRAL [37], thereby suggesting that GDF15 signaling is independent of GFRAL in muscle cells. The peripheral effects of GDF15 have recently been confirmed in other cells and organs that do not express GFRAL [38,39]. It has been proposed that the peripheral effects of GDF15 are mediated by the secretion of the other forms of GDF15 which differ from the dimer, such as monomeric pro-GDF15 or dimeric pro-GDF15 [10]. Future studies are needed to uncover the peripheral receptors/pathways mediating the effects of GDF15 that do not involve GFRAL.

Glossary

Adipokine (adipocytokine): a peptide produced by adipose tissue that has an autocrine, paracrine, or endocrine function.
Chronic low-level inflammation: obesity-induced inflammation that differs from inflammation in classical immunity through being low grade and producing much lower levels of circulating cytokines. It is also considered to be a chronic inflammation because a relatively long treatment with a high-fat diet is required (>8 weeks in animal models) before inflammation is observed in the adipose tissue. Obesity-induced inflammation results from the exposure to an excess of nutrients, resulting in adipocyte hypertrophy, macrophage infiltration and polarization, and the activation of inflammatory pathways.
Cytokine: a small protein secreted by cells that has a specific effect on the interactions and communication between cells.
Diabetic nephropathy (diabetic kidney disease): chronic loss of kidney function caused by diabetes. It is the most common cause of chronic kidney disease and end-stage renal disease.
Endoplasmic reticulum (ER) stress: the result of any stimulus that provokes the accumulation of misfolded proteins in the lumen of the ER. ER stress triggers the unfolded protein response, an adaptive (defensive) ER-stress response that involves the activation of a signaling pathway to restore folding capacity. If ER homeostasis is not restored, inflammation and apoptosis are induced.
Glucolipotoxicity: the deleterious effects of elevated glucose and fatty acid levels.
Integrated stress response (ISR): an adaptive cytoprotective signaling pathway activated by different stimuli (including oxidative stress, ER stress, hypoxia, nutrient deprivation, and mitochondrial stress) that restores balance by downregulating protein synthesis and reprogramming gene expression.
Metformin: the first-line drug to be prescribed following the diagnosis of T2DM in the absence of contraindications such as severe renal or hepatic insufficiency. It is one of the few oral drugs for T2DM that promotes weight loss rather than weight gain.
Mitochondrial dysfunction: loss of efficiency in the electron transport chain

GDF15 in T2DM

Clinical studies have reported that an elevated GDF15 level is a useful biomarker for identifying healthy versus prediabetic subjects at a higher risk of progressing to diabetes [40]. Similar to what has been reported in obesity, an increase in the endogenous levels of GDF15 in T2DM can be viewed as a cell response to restore glucose homeostasis. However, this increase in endogenous GDF15 levels does not seem to be sufficient to prevent metabolic alterations, and only the administration of supraphysiological levels of exogenous GDF15 in animal models of obesity-induced T2DM has been shown to ameliorate the metabolic alterations [36]. In the next sections of this review, the beneficial effects of GDF15 in the treatment of T2DM and its potential pharmacological modulation will be discussed.

Effects of GDF15 on β cells

As mentioned earlier, hyperglycemia occurs when the defective insulin secretion by β cells can no longer overcome peripheral IR, leading to glucolipotoxicity, which accelerates β cell death by apoptosis and the decline in insulin secretion. Interestingly, it has been reported that exposure of human islets and human β cells to an inflammatory milieu reduces GDF15 levels by blocking the translation of its transcript, whereas preincubation of human islets with rGDF15 ameliorates proinflammatory cytokine-mediated β cell apoptosis and rGDF15 administration reduces the incidence of diabetes in nonobese diabetic (NOD) mice [41]. These effects of GDF15 are independent of GFRAL, since human islets do not express this receptor. These findings are inconsistent with those of another study showing that **endoplasmic reticulum (ER) stress**-induced apoptosis is reduced in islets isolated from *Gdf15* knockout mice [42]. Moreover, *Gdf15* deletion mitigates streptozotocin-induced diabetes by preserving β cells and insulin levels. These conflicting findings warrant further studies to clearly decipher the role of GDF15 in β cell apoptosis.

GDF15 as a myokine

Elevated *GDF15* expression in skeletal muscle has been reported under conditions of muscle-specific **mitochondrial dysfunction** in humans and different mouse models [34,35]. In fact, it has been proposed that the increased production and secretion of GDF15 by skeletal muscle upon mitochondrial stress regulate systemic metabolism, converting GDF15 into a key **myomitokine** [34]. This study reported that mice with a skeletal-muscle-specific deficiency of *Crif1* (also known as *Gadd45gip1*), with decreased oxidative phosphorylation (OxPhos) function, displayed increased circulating levels of GDF15 (<400 pg/ml) that protected these mice from HFD-induced obesity and IR [34]. In another animal model with a compromised muscle-specific mitochondrial OxPhos capacity, genetic ablation of *Gdf15* promoted fat mass expansion and a reduction in the synthesis of high-energy molecules such as ATP. It is characteristic of aging and, essentially, of all chronic diseases.

Myokine: a peptide or protein released from skeletal muscle cells that has an autocrine, paracrine, or endocrine function.

Myomitokine: a stress-response molecule secreted from skeletal muscle in response to mitochondrial stress.

Nonalcoholic fatty liver disease (NAFLD): a pathological entity that ranges from isolated steatosis or NAFL (defined as the presence of cytoplasmic triglyceride droplets in more than 5% of hepatocytes with no evidence of hepatocellular injury) to nonalcoholic steatohepatitis (NASH).

Nonalcoholic steatohepatitis (NASH): the most severe stage of NAFLD, defined as the presence of hepatic steatosis with inflammation and hepatocyte injury and different degrees of fibrosis.

Transforming growth factor β (TGF-β): a secreted cytokine that plays an important role in wound healing, angiogenesis, immunoregulation, and cancer.

Box 1. Regulation of GDF15

GDF15 was discovered in 1997 as a cytokine secreted by activated macrophages; it was originally named macrophage inhibitory cytokine-1 [86–88]. Shortly after, it was discovered in other cells and tissues and received additional names (NSAID-activated gene, placental bone morphogenetic protein, placental transforming growth factor-β and prostate-derived factor). Although GDF15 was initially classified as a divergent member of the TGF-β superfamily, the recent discovery that GDF15 binds to the glial cell line-derived neurotrophic factor (GDNF)-like α-1 (GFRAL)/RET coreceptor complex suggests that this cytokine could be reclassified as a member of the GDNF family [89,90]. GDF15 is expressed in several tissues such as the placenta, kidneys, liver, skeletal muscle, adipose tissue, lungs, pancreas, heart, gut, and brain. It is synthesized in the cytoplasm as a propeptide monomer (pro-GDF15) (~40 kDa), which dimerizes to form pro-GDF15 (~80 kDa), and subsequently undergoes cleavage to be secreted as the mature dimer of ~30 kDa [10] (Figure I). Interestingly, pro-GDF15 has been reported to inhibit the binding of the small mothers against decapentaplegic (SMAD) complex to its response elements in the promoter of its target genes, thus attenuating the TGF-β1-induced SMAD signaling and thereby affecting cell migration and invasion [91]. The elevated GDF15 levels in cellular stress may be the result of the activation of different transcription factors, including p53, Krüppel-like factor-4 (KLF4), early growth response protein 1 (EGR1), hypoxia-inducible factor-1α (HIF-1α), nuclear factor erythroid 2–related factor 2 (Nrf2), activating transcription factor 3 (ATF3) and ATF4, and C/EBP homologous protein (CHOP) [11,38,78,90,92] (Figure I). Among these transcription factors, CHOP plays a key role in upregulating GDF15 expression via the ISR, a signaling pathway that aims to restore cellular homeostasis in response to different stresses (oxidative stress, ER stress, hypoxia, nutrient deprivation and mitochondrial stress). To recover from these stresses, cells activate this adaptive response through the phosphorylation of eukaryotic initiation factor-2α (eIF2α), which increases ATF4 activity, ultimately leading to increased CHOP levels. Both ATF4 and CHOP are key regulators of *Gdf15* expression [11]. Overall, these findings suggest that GDF15 is a stress-induced cytokine upregulated by the ISR pathway that acts as an endocrine signal to restore cellular homeostasis.

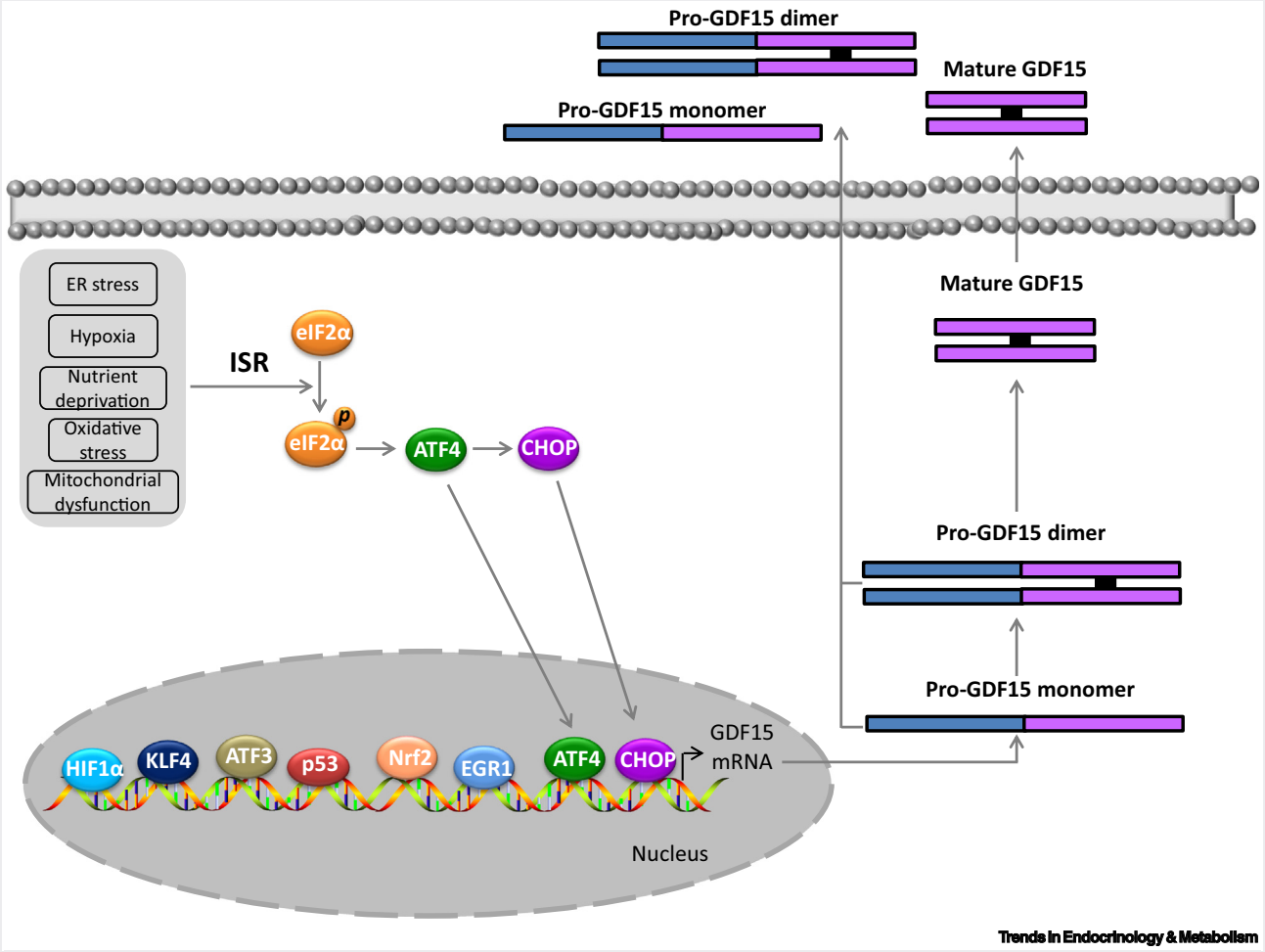

Figure I. Schematic of growth differentiation factor 15 (GDF15) regulation and secretion. Different stimuli such as endoplasmic reticulum (ER) stress, hypoxia, nutrient deprivation, oxidative stress, and mitochondrial dysfunction activate the integrated stress response (ISR) pathway, which ultimately causes an upregulation in the expression of *GDF15*. Once activated, the ISR leads to the phosphorylation of eukaryotic initiation factor-2α (eIF2α), which increases activating transcription factor 4 (ATF4) activity, thereby causing an increase in CCAAT-enhancer-binding protein homologous protein (CHOP) levels. These two transcription factors seem to be of critical importance in the upregulation of *GDF15* expression. Other transcription factors – including the stress-responsive transcription factors p53 and EGR1, as well as nuclear factor erythroid 2-related factor 2 (Nrf2), ATF3, Krüppel-like factor-4 (KLF4), and hypoxia-inducible factor-1α (HIF-1α) – have been reported to be key regulators of *GDF15* expression. GDF15 is synthesized in the cytoplasm as a propeptide monomer (pro-GDF15), which dimerizes into a dimeric pro-GDF15 and undergoes cleavage to form the mature dimer. The three indicated forms are probably secreted. Abbreviation: EGR1: early growth response protein 1.

and abolished white adipose tissue browning and insulin sensitivity [35]. These studies suggest that GDF15 protects against metabolic alterations caused by mitochondrial dysfunction, thereby preventing the hypothesized development of obesity and IR in these states [43]. In addition, these studies demonstrate that muscle-derived GDF15 might result in systemic metabolic effects through GFRAL or peripheral receptors that are as yet unknown. It is important to highlight that a previous study reported that mice fed an HFD showed increased serum GDF15 levels from 100 to approximately 300 pg/ml, with *Gdf15* expression being upregulated in the liver and brown and epididymal adipose tissue, but not in the skeletal muscle, kidneys or subcutaneous adipose tissue [11]. The increase in GDF15 levels caused by the HFD was not sufficient to prevent the development of obesity and glucose intolerance [20]. These data raise several issues. As

mentioned earlier, it seems that physiological increases (two- to threefold) of endogenous GDF15 levels are not sufficient to prevent the metabolic alterations associated with obesity, which might be the result of a state of GDF15 resistance, reinforcing the role for MT1-MMP inhibitors in the treatment of obesity and associated diseases such as T2DM. In addition, considering that an HFD induces *GDF15* expression in only a restricted number of tissues, the primary source of circulating GDF15 and the proportion of the different forms of this cytokine released into the plasma by these tissues might result in different metabolic effects. Therefore, it remains to be studied whether the pharmacological modulation of GDF15 derived from skeletal muscle produces systemic metabolic effects that may help to treat obesity and T2DM.

Like GDF15, fibroblast growth factor 21 (FGF21) has also been reported to function as a **myokine** that is released upon mitochondrial dysfunction. However, FGF21 has been documented to be dispensable for systemic metabolic adaptations in response to muscle mitochondrial stress [44]. Furthermore, it has been considered a biomarker for mitochondrial diseases, although with a lower diagnostic sensitivity and specificity than GDF15 [45].

GDF15 in adipose tissue

Mice overexpressing human *GDF15* display reduced white and brown adipose tissue together with improved glucose tolerance, lower insulin levels, and resistance to obesity despite equivalent food intake [24]. These transgenic mice also exhibit an increased expression of thermogenic genes in brown adipose tissue and an increased expression of lipolytic genes in white and brown adipose tissues, suggesting higher energy metabolism.

As mentioned earlier, exogenous GDF15 administration preferentially suppresses appetite for an HFD, with a recent study confirming that GDF15 secreted by adipose tissue acts as an **adipokine (adipocytokine)** that mediates this effect [46]. In that study, transgenic mice with adipocyte-specific **integrated stress response (ISR)** activation displayed increased *Gdf15* expression via activating transcription factor 4 (ATF4) and CCAAT-enhancer-binding protein homologous protein (CHOP) that preferentially inhibited HFD intake, thereby decreasing body weight and improving the glucose tolerance and obesity induced by the HFD. GFRAL deficiency abrogated the preferential inhibition of HFD intake and the reduction in obesity caused by ISR activation in adipose tissue, suggesting the involvement of GDF15.

IR in obesity is closely related to adipose tissue inflammation, caused mainly by macrophage infiltration of the adipose tissue in obese rodents and humans [47]. Furthermore, defects in autophagy – a lysosomal degradation process that removes long-lived and misfolded proteins and damaged organelles, in addition to regulating growth and metabolism – are involved in the etiology of T2DM [48]. Autophagy is under the control of several transcription factors, including transcription factor EB (TFEB), a master regulator of autophagy-related gene expression and lysosomal biogenesis [49]. Remarkably, one study has reported that *Tfeb* expression is upregulated in the adipose tissue macrophages of obese mice and humans, while macrophage-specific overexpression of *Tfeb* in mice protects against diet-induced obesity, adipose tissue inflammation, and IR by inducing GDF15 [50]. Of note, TFEB-induced GDF15 prevents diet-induced obesity and IR by enhancing lipid catabolism and reducing adipose tissue inflammation without affecting food intake. The authors of that study proposed that activation of the TFEB–GDF15 axis could act as a lysosomal stress response that could play crucial roles in regulating obesity-associated metabolic diseases.

Similar to the studies conducted on skeletal muscle, adipocyte-specific *Crif1* knockout mice with a decreased adipocyte OxPhos function fed an HFD were reported to be resistant to obesity,

exhibited higher energy expenditure, and showed improved glucose tolerance [51]. These changes were accompanied by an increased expression and secretion of FGF21 and GDF15. To explore the roles of these cytokines in this animal model, adipocyte-specific *Crif1* knockout mice were generated with a global deletion of either *Gdf15* or *Fgf21*. After exposure of these models to an HFD for 8 weeks, it could be concluded that GDF15 attenuated the progression of obesity by increasing energy expenditure, whereas FGF21 ameliorated HFD-induced IR and obesity without affecting energy expenditure.

GDF15 is also an adipokine released by brown and beige cells in response to thermogenic stimuli, acting on macrophages to mitigate proinflammatory signaling [52]. Further studies have confirmed a role for GDF15 in inflammation in white adipose tissue. Transgenic mice ubiquitously overexpressing the human *GDF15* gene exhibited reduced levels of the NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP3) inflammasome in white adipose tissue, and also showed decreased levels of interleukin (IL)-18, IL-1β, tumor necrosis factor α (TNF-α) expression, and markers of macrophage infiltration [22]. Consistent with this, the administration of rGDF15 reduced the markers of inflammation in serum [53], while the administration of a neutralizing antibody against GDF15 to obese mice increased the levels of inflammatory markers in adipose tissue [54]. Likewise, mice with a reduced mitochondrial OxPhos function through a myeloid-specific deletion of *Crif1* showed decreased levels of GDF15 in macrophages, which was accompanied by adipose tissue inflammation and IR [55]. The administration of rGDF15 improved macrophage OxPhos function and reversed adipose tissue inflammation and IR. Moreover, the administration of IL-4 to wild-type mice fed an HFD reduced body weight and plasma insulin levels and improved glucose intolerance, but these actions were absent in *Gdf15*$^{-/-}$ mice, suggesting that the metabolic actions of IL-4 depend on GDF15. Likewise, the administration of IL-13 to wild-type mice fed an HFD improved glucose intolerance, but this effect was not observed in *Gdf15*$^{-/-}$ mice [56]. Finally, GDF15 is required for mitigating aging-mediated local and systemic inflammation by reducing the activation of resident immune cells in metabolic organs such as the adipose tissue and others, thereby contributing to maintaining glucose homeostasis and insulin sensitivity in humans and mice [57].

Overall, these findings indicate that GDF15 has a role in preventing inflammation and, thereby, contributes to reducing IR, although further studies are needed to establish the molecular mechanisms responsible for these anti-inflammatory effects.

Renoprotective effects of GDF15

GDF15 has also been reported to protect against **diabetic nephropathy (diabetic kidney disease)**, since deletion of *Gdf15* increases renal tubular and interstitial damage in animal models of both type 1 diabetes mellitus (T1DM) and T2DM [58]. In both models, *Gdf15* deletion increased the levels of fibrotic markers, suggesting that GDF15 may play an antifibrotic role in the diabetic kidney. In addition, in the T1DM model, *Gdf15*$^{-/-}$ mice exhibited increased levels of inflammatory markers, whereas impaired kidney function was observed in the T2DM model [58]. A more recent study has confirmed that GDF15 attenuates damage and inflammatory response in the kidney and provides additional evidence on the renoprotective effect of GDF15 [59]. This study also indicated that part of the renoprotective effects of GDF15 may result from its ability to preserve the renal levels of Klotho, a receptor implicated in the pathophysiology of chronic kidney disease. These findings support GDF15 as a promising therapeutic target for diabetic kidney disease, but the mechanisms involved remain to be clearly established.

GDF15 in NAFLD

NAFLD ranges from steatosis to **nonalcoholic steatohepatitis (NASH)**. The latter is a more advanced-stage condition that is distinguished from simple steatosis by the presence of

inflammation, hepatocyte damage, and different degrees of fibrosis. NASH confers a higher risk of progression to advanced fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). GDF15 levels are elevated in humans with NAFLD, with the levels being higher the more the disease has advanced [60]. For this reason, it has been proposed that GDF15 serum levels are a novel biomarker for advanced fibrosis in NAFLD. *Gdf15* deletion exacerbates hepatic steatosis, inflammation, and fibrosis in mice fed a NASH-inducing diet, whereas these phenotypes are improved in mice overexpressing human *GDF15* [61]. One of the NASH models used to study this was the methionine/choline-deficient (MCD) diet model, a widely used dietary NASH model that does not induce obesity, thereby indicating that the increase in GDF15 was not caused by increased adiposity. In this NASH model, there was an increase in the levels of the transcription factors ATF4 and CHOP in the liver, suggesting a possible implication of these factors in the increase of GDF15. The beneficial effects of GDF15 in NAFLD have been linked to its capacity to increase fatty acid β-oxidation, thereby attenuating hepatic steatosis progression [62], as well as to its antifibrotic effects in the liver and in hepatic stellate cells [61]. More recently, it was reported that *GDF15* overexpression in transgenic mice fed an HFD alleviated obesity and hepatic steatosis, increased fatty acid oxidation and lipolysis, inhibited fatty acid synthesis and uptake, and attenuated the activation of the absent in melanoma 2 (AIM2) inflammasome and the secretion of IL-18 and IL-1β [63]. All these changes were observed in the absence of changes in food intake. The decrease in the activation of the AIM2 inflammasome caused by GDF15 might be mediated by reductions in lipid-induced oxidative stress and in mitochondrial damage and the subsequent decrease in the release of double-stranded DNA (dsDNA) into the cytosol. This indicates that, in addition to its effects on lipid homeostasis, GDF15 ameliorates hepatic steatosis by reducing oxidative stress and AIM2 inflammasome activation.

Despite these beneficial effects of GDF15 in NAFLD, GDF15 has been reported to act as an HCC-derived immunosuppressive molecule. Supporting this role, a neutralizing antibody against GDF15 was shown to effectively eradicate HCC and augment antitumor immunity in mice [38]. This immunomodulatory function of GDF15 was independent of GFRAL and was mediated by the interaction of GDF15 with the CD48 receptor on T cells, a receptor expressed only on leukocytes. These results suggest that a GDF15-neutralizing antibody might be a potential therapeutic tool for the treatment of HCC.

Collectively, these findings suggest that GDF15 has a direct impact on the liver, without reducing food intake or involving GFRAL, since this receptor is not expressed in this organ. Further studies are required to establish the receptor/pathways mediating the effects of GDF15 in NAFLD as well as its potential role in HCC.

GDF15 and physical activity

Exercise helps to prevent T2DM by lowering blood glucose levels and improving insulin sensitivity throughout the body [64]. Moderate exercise has been reported to cause modest increases in serum GDF15 levels in humans [36,65]. Long-term endurance exercise (e.g., marathon running) leads to circulating GDF15 levels (1500–2000 pg/ml) similar to those observed in some diseases (cancer or mitochondrial disease with GDF15 levels >2000 pg/ml), but after the exercise GDF15 returns to basal levels in 24 h [66]. The role of GDF15 secretion in exercise is not well known, but acute administration of GDF15 reduced voluntary running in mice, although it did not affect forced treadmill running [36]. In addition, the secretion of GDF15 by a contracting skeletal muscle triggers adipose tissue lipolysis to sustain fuel availability during exercise [67]. However, the tissue/organ responsible for increasing the circulating GDF15 levels in exercise remains to be established. The liver, heart, and skeletal muscle in mice show increased *Gdf15* expression after exercise [36]. In humans, GDF15 levels are similar in plasma samples from the femoral artery and femoral vein before and after 1 h of vigorous exercise, suggesting that skeletal muscle does

not contribute to the increase in GDF15 levels in these conditions [65]. Future studies are required to establish what organ/tissue is responsible for the secretion of GDF15 in exercise, and whether the increase in the levels of this cytokine in exercise contributes to the prevention of T2DM.

Pharmacological modulation of GDF15

Metformin is the most prescribed drug for the treatment of T2DM, although its precise mechanism of action remains only partially known [68]. In the liver, metformin attenuates gluconeogenesis by activating AMPK, which inhibits the expression of genes involved in this process [68]. At pharmacological doses, metformin directly activates AMPK, while at supra-pharmacological doses it also indirectly activates this kinase through the inhibition of complex I of the mitochondrial electron transport chain [68]. The beneficial effects of metformin also extend to the skeletal muscle, gut, and kidneys [69]. In 2017, a clinical trial revealed that metformin increased the serum levels of GDF15 in a dose-dependent manner; the authors proposed that GDF15 was a biomarker for the response to this drug [70]. More recently, two studies reported that the increase in GDF15 levels caused by metformin contributed to its effects on food intake and weight gain [71,72] (see Figure 1 in main text). The first of these studies demonstrated that metformin increased serum GDF15 levels and that this increase was associated with reductions in body mass in subjects with T2DM [71]. In addition, metformin increased the expression and secretion of GDF15 in primary cultures of mouse hepatocytes through the ISR pathway and the involvement of CHOP and ATF4. By contrast, AMPK did not seem to be involved, since metformin upregulated *Gdf15* expression in hepatocytes isolated from AMPKβ1-null mice. Moreover, oral metformin increased serum GDF15 levels and also reduced food intake, body mass, and glucose intolerance in HFD-fed wild-type mice, but these effects were abrogated in *Gdf15*$^{-/-}$ mice [71]. The second study reported that the administration of metformin to human subjects without diabetes for 2 weeks caused a 2.5-fold increase in serum GDF15 levels, while longer treatments led to a sustained increase in the circulating levels of this cytokine [72]. In wild-type mice, oral metformin elevated circulating GDF15 levels, but in that study metformin mostly induced *Gdf15* expression in the distal intestine, colon, and kidneys, suggesting that these tissues, and not the liver, could be the source contributing to the increase in circulating GDF15 levels. Metformin reduced food intake in HFD-fed wild-type mice, but not in mice lacking GDF15 or GFRAL or in mice treated with a GFRAL-antagonist antibody. Despite the robust findings of those two studies, a recent work challenged these effects of metformin, reporting that although metformin increased GDF15 levels, the GDF15–GFRAL pathway was dispensable for the effects of metformin on the energy balance [73]. Likewise, it has been reported that AMPK upregulates GDF15 [37,74], which is in conflict with the findings of a previous study [71]. In fact, the AMPK activator, A769662, increased hepatic GDF15 levels independently of CHOP [74]. In that same study, the pharmacological activation of AMPKβ1-containing complexes increased GDF15 levels, whereas liver *Gdf15* expression and serum GDF15 levels were attenuated in mice lacking AMPKβ1-containing complexes [74]. Future studies are needed to explain these discrepancies.

We also previously reported that GDF15 plays a key role in mediating the metabolic effects of peroxisome proliferator-activated receptor β/δ (PPARβ/δ) agonists by activating the AMPK–p53 pathway [37]. This PPAR isotype regulates glucose and lipid metabolism, as well as inflammation. Consequently, its activation by ligands mitigates dyslipidemia and hyperglycemia, improves whole-body insulin sensitivity, and prevents diet-induced obesity [75,76]. We previously observed that pharmacological PPARβ/δ activation increased GDF15 levels, ameliorated glucose intolerance, fatty acid oxidation, ER stress, and inflammation, and activated AMPK in HFD-fed mice, and these effects were abrogated by the injection of a GDF15-neutralizing antibody and in *Gdf15*$^{-/-}$ mice [37]. As mentioned earlier, the fact that GDF15 activates AMPK in cultured myotubes and isolated skeletal muscle demonstrates that this effect does not involve GFRAL.

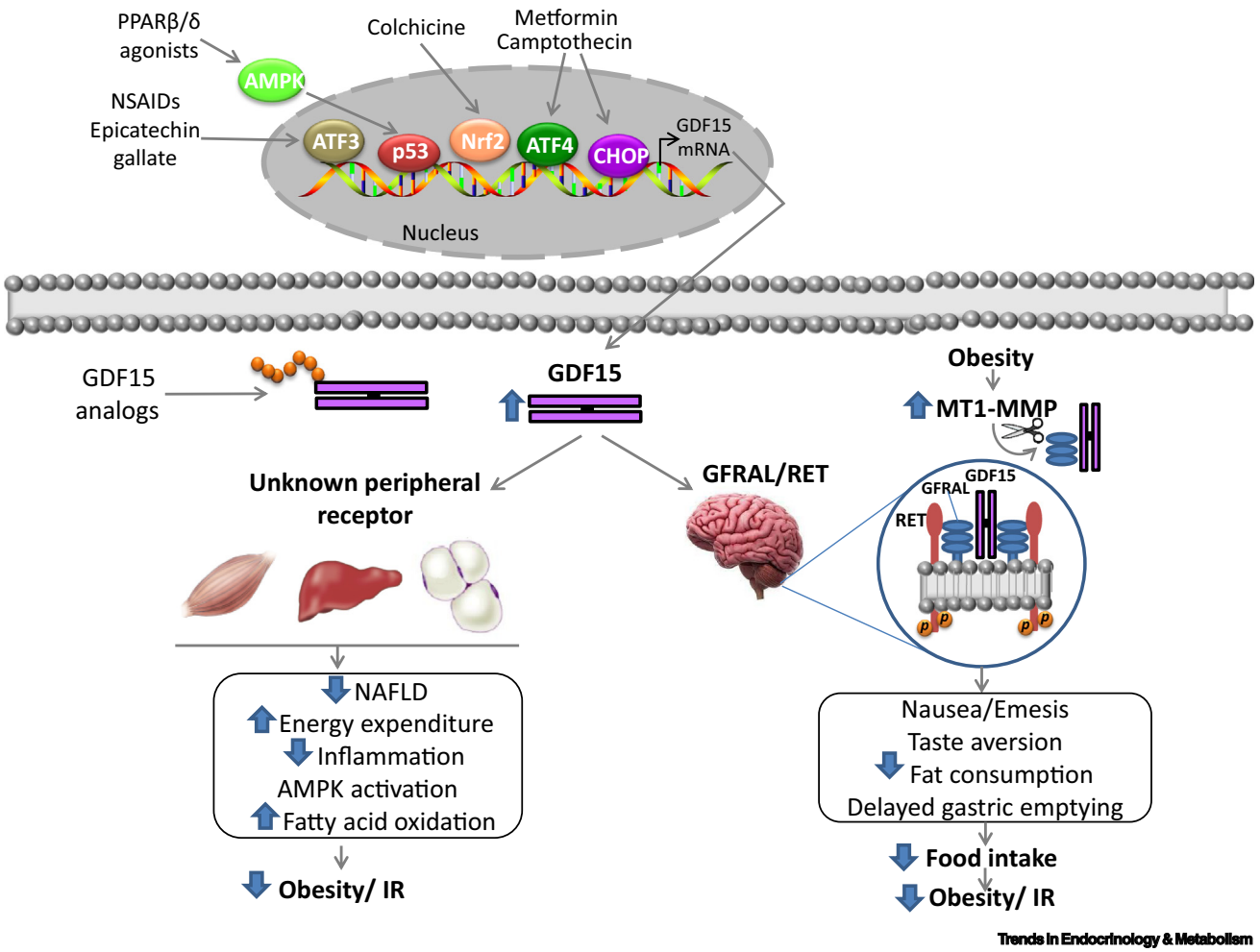

Figure 1. Schematic showing the pharmacological regulation of growth differentiation factor 15 (GDF15)and its main effects in obesity and type 2 diabetes mellitus (T2DM). Metformin, peroxisome proliferator-activated receptor (PPAR) β/δ agonists, colchicine, camptothecin, nonsteroidal anti-inflammatory drugs (NSAIDs), and polyphenols (such as epicatechin gallate) have been reported to increase the expression and circulating levels of GDF15. The binding of this cytokine to its central receptor glial cell line-derived neurotrophic factor (GDNF)-like alpha-1 (GFRAL) suppresses appetite by inducing nausea/emesis, taste aversion, reduced fat consumption, and delayed gastric emptying. These central effects of GDF15 reduce obesity and improve T2DM. Peripheral effects of GDF15 not involving GFRAL have also been reported through an unknown receptor that mediates different effects ameliorating obesity and insulin resistance. Approaches to potentiate the GDF15–GFRAL pathway may include the use of GDF15 analogs or inhibitors of membrane-bound matrix metalloproteinase 14 (MT1-MMP), a metalloproteinase that inactivates GFRAL and seems to be increased in obesity, causing resistance to the anorexigenic effects of GDF15. Abbreviations: AMPK, AMP-activated protein kinase; ATF, activating transcription factor; CHOP, CCAAT-enhancer-binding protein homologous protein; IR, insulin resistance; NAFLD, nonalcoholic fatty liver disease; Nrf2, nuclear factor erythroid 2-related factor 2; RET, rearranged during transfection.

These findings indicate that GDF15 upregulation contributes to the antidiabetic beneficial effects of PPARβ/δ agonists.

Other drugs have been reported to increase the levels of GDF15, which is likely to mediate at least some of their effects, pointing to this cytokine as a target for the treatment of obesity and T2DM as well as other pathologies. One of the initial names given to GDF15 was nonsteroidal anti-inflammatory drug (NSAID)-activated gene (NAG-1) because it was upregulated by NSAIDs that were mainly known as inhibitors of cyclooxygenase (COX) [77]. This upregulation of GDF15 by NSAIDs was observed in colorectal cancer cell lines, many of which did not express COX enzymes. It was recently

shown that the activation of Nrf2 by a subset of NSAIDs mediates the increase in GDF15 levels in myeloid cells [78]. Likewise, the old anti-inflammatory drug colchicine has been shown to increase the secretion of GDF15 in hepatocytes via Nrf2 activation [38], while circulating GDF15 is required for the anti-inflammatory effect of colchicine *in vivo*. In fact, GDF15 acts as a communication molecule between hepatocytes and myeloid cells, sustaining the activation of the latter. This effect of colchicine is not mediated by GFRAL, since myeloid cells do not express this receptor. Moreover, the effect of endogenous GDF15 *in vivo* is not reproduced by mature rGDF15, leading to the proposal that the molecular form of GDF15 released by colchicine and the receptor on myeloid cells mediating its effects are new and require further characterization. In addition, it has been suggested [78] that canagliflozin, an inhibitor of sodium–glucose cotransporter-2 (SGLT2i), used in the treatment of T2DM, produces antioxidant and anti-inflammatory effects via Nrf2 [79,80], thereby suggesting that this antidiabetic drug might upregulate GDF15 through this transcription factor. In line with this, another SGLT2i, empagliflozin, promotes Nrf2 nuclear translocation [81] and increases circulating GDF15 levels in humans [82].

Camptothecin, a drug with potential antitumor activity, reduces obesity by inducing GDF15 [83]. This drug increases circulating GDF15 levels in diet and genetic models of obesity by upregulating *Gdf15* expression predominantly in the liver through the activation of the ISR pathway. Due to its anorectic effect, the increase in GDF15 levels caused by camptothecin reduces food intake, body weight, blood glucose, and liver fat in wild-type mice, but not in GFRAL-deficient mice. Finally, the green tea polyphenol epicatechin gallate, which may exert beneficial effects in diabetes and is an AMPK activator [84], upregulates *Gdf15* expression via an ATF3-binding site in its promoter [85].

Box 2. Potential therapeutic applications of GDF15-based therapies in obesity and T2DM

The function of GDF15 as an appetite suppressor and a regulator of energy metabolism has raised the possibility of pharmacologically administering GDF15 to treat obesity and T2DM [93]. However, the pharmacokinetic and physicochemical properties of native GDF15 present several challenges for its development as a drug, such as its subcutaneous administration, short half-life (~3 h in mice and nonhuman primates), high aggregation propensity, and susceptibility to the proteases in serum [94]. This has led to the development of GDF15 analogs that aim to improve the efficacy and pharmacokinetics of GDF15 for the treatment of obesity and T2DM. Some of the strategies used to obtain long-lasting GDF15 analogs include fusing this cytokine with an immunoglobulin Fc domain [32]. One of these Fc–GDF15 fusion molecules has been observed to reduce body weight and improve metabolic parameters, including glucose intolerance, in obese mice and obese cynomolgus monkeys [32]. Another approach to prolonging the effects of GDF15 has been to fuse this cytokine with human serum albumin (HSA). The subcutaneous administration of one of these analogs with a half-life longer than 8 days has been reported to reduce food intake in nondiabetic cynomolgus monkeys with spontaneous obesity [18]. Interestingly, no signs of nausea, malaise, or emesis have been observed in any of the monkeys. Finally, some of these GDF15 analogs have entered clinical trials, including long acting (LA)-GDF15 (Novo Nordisk), the GDF15 agonist LY-3463251 (Lilly), and JNJ-9090/CIN-109 (Jansenn/CinFinaPharma). Another potential strategy to target the GDF15–GFRAL pathway involves the use of small molecules such as inhibitors of MT1-MMP, the metalloproteinase that inactivates GFRAL and may be responsible for the development of resistance to the anorexigenic effects of GDF15 in obesity [33]. Of note, the combination of a GDF15 analog and an MT1-MMP inhibitor could reduce the dose of the former in the treatment of obesity and T2DM, thereby attenuating potential side effects. In addition, a recent study extended the potential applications of MT1-MMP inhibitors beyond GDF15, since this metalloproteinase is a central regulator of insulin sensitivity during aging [95]. That study reported that the increase in MT1-MMP levels during aging in several tissues suppresses insulin signaling by cleaving the insulin receptor, with the inhibitors of this enzyme restoring insulin receptor levels and insulin sensitivity in aged mice. In young mice, MT1-MMP overexpression in the liver reduces the levels of the insulin receptor and impairs hepatic insulin sensitivity [95].

Considering that many drugs increase endogenous GDF15 levels through the activation of several pathways, strategies to upregulate circulating GDF15 levels by targeting these pathways show promise. During the development of GDF15-based drugs, special attention should be paid to the potential side effects of GDF15, mainly nausea, vomiting, and tumorigenicity. It has been reported that elevated levels of GDF15 in mice fed an HFD for a year did not result in detrimental effects in many organs and tissues [41], suggesting that high levels of endogenous GDF15 are safe in mice. Ultimately, only clinical studies in humans can assess whether GDF15-based therapies exhibit efficacy and safety in the treatment of obesity and T2DM.

Collectively, these data indicate that the pharmacological modulation of GDF15 contributes to the antidiabetic effect of several drugs, including metformin, indicating that the upregulation of this cytokine is potentially safe and shows efficacy in reducing body weight and T2DM (Box 2). Antidiabetic drugs increase GDF15 levels via several transcription factors, including the ISR factors CHOP and ATF4, Nrf2, and AMPK. However, further studies are required to explore additional unknown mechanisms by which antidiabetic drugs may upregulate GDF15 levels.

Concluding remarks and future prospects

T2DM is a complex chronic disease characterized by the presence of hyperglycemia that results from a combination of IR and β cell failure. There is compelling evidence that GDF15 may be an attractive target for the treatment of T2DM. In fact, GDF15 and its analogs reduce food intake and body weight, and also attenuate inflammation, hepatic fat deposition and glucose intolerance. In addition, the increase in circulating GDF15 levels by antidiabetic drugs such as metformin has shed light into some of its effects on energy metabolism. The discovery of GFRAL as the cognate central receptor for GDF15 explains its suppressive effects on appetite, but new studies indicating that GDF15 produces peripheral effects through unknown receptors open up new exciting pathways to target energy metabolism in the treatment of obesity and T2DM. However, safety concerns, including those around nausea and emesis (see Outstanding questions), have been raised for GDF15, with questions still remaining regarding the safety of chronic treatment with GDF15-based drugs.

Outstanding questions

What is the peripheral receptor mediating the antidiabetic effects of GDF15?

Will GDF15-based drugs show an improvement in clinical outcomes of patients with obesity and T2DM?

How would potential side effects affect the development of GDF15-based therapies for the treatment of obesity and T2DM?

Is GDF15 involved in the antidiabetic effects of metformin?

Are pharmacological approaches targeting MT1-MMP an option for the treatment of obesity and T2DM?

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Declaration of interests

No interests are declared.

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