GDF15: emerging biology and therapeutic applications for obesity and cardiometabolic disease

Dongdong Wang[1], *Emily A. Day*[1], *Logan K. Townsend*[1], *Djordje Djordjevic*[2], *Sebastian Beck Jørgensen*[2] *and Gregory R. Steinberg*[1] ✉

Abstract | Growth differentiation factor 15 (GDF15) is a member of the TGFβ superfamily whose expression is increased in response to cellular stress and disease as well as by metformin. Elevations in GDF15 reduce food intake and body mass in animal models through binding to glial cell-derived neurotrophic factor family receptor alpha-like (GFRAL) and the recruitment of the receptor tyrosine kinase RET in the hindbrain. This effect is largely independent of other appetite-regulating hormones (for example, leptin, ghrelin or glucagon-like peptide 1). Consistent with an important role for the GDF15–GFRAL signalling axis, some human genetic studies support an interrelationship with human obesity. Furthermore, findings in both mice and humans have shown that metformin and exercise increase circulating levels of GDF15. GDF15 might also exert anti-inflammatory effects through mechanisms that are not fully understood. These unique and distinct mechanisms for suppressing food intake and inflammation makes GDF15 an appealing candidate to treat many metabolic diseases, including obesity, type 2 diabetes mellitus, non-alcoholic fatty liver disease, cardiovascular disease and cancer cachexia. Here, we review the mechanisms regulating GDF15 production and secretion, GDF15 signalling in different cell types, and how GDF15-targeted pharmaceutical approaches might be effective in the treatment of metabolic diseases.

Obesity is a global health problem driving the development of cardiometabolic diseases, including type 2 diabetes mellitus (T2DM), non-alcoholic fatty liver disease (NAFLD) and cardiovascular diseases (CVDs)[1,2]. Obesity causes NAFLD and insulin resistance, both of which are precursors to the development of T2DM[3]. NAFLD can also progress to non-alcoholic steatohepatitis (NASH), the leading cause of cirrhosis and hepatocellular carcinoma[4]. Collectively, T2DM and NAFLD are important risk factors for CVDs, the leading cause of death globally[5]. Although dietary and lifestyle interventions can be effective at reducing obesity, they have low rates of long-term success[6]. As such, pharmacotherapies have been developed for treating obesity but, to date, these agents typically result in about 5–8% weight loss, which might not be sufficient to correct obesity-related comorbidities in some individuals[7]. Thus, an urgent need exists for novel obesity therapeutics to treat cardiometabolic disease.

Growth differentiation factor 15 (GDF15) is a distant member of the transforming growth factor-β (TGFβ) superfamily that circulates as a ~25 kDa homodimer consisting of two 112 amino acid polypeptide chains, which are linked by a single inter-chain disulfide bond[8]. Several tissues abundantly express GDF15, including liver[9], intestine[10], kidneys[11] and placenta[12]. Under normal physiological conditions, circulating levels of GDF15 are in the range of 0.1–1.2 ng/ml (REFS[13,14]); however, levels of GDF15 are markedly elevated in humans during cellular stress such as in cardiac and renal failure, chronic liver disease, various cancers, chronic inflammatory diseases (for example, rheumatoid arthritis) and mitochondrial diseases[8,15]. Interestingly, obesity is also associated with increased serum concentrations of GDF15, which are also positively correlated with body weight and adipose tissue mass[16] even after correcting for age and sex[17,18]. GDF15 concentrations are also higher in obese mice and rats compared with lean controls[18,19]. However, more detailed analysis of the GDF15–body mass relationship in monozygotic twins without obesity has found that serum levels of GDF15 are inversely correlated with body mass index (BMI)[20]. These data suggest that increased GDF15 is associated with decreased body mass and the increases observed in obesity might be a consequence of obesity rather than a cause. Consistent with this concept, circulating levels of GDF15 are increased in response to

[1]*Centre for Metabolism, Obesity and Diabetes Research and the Department of Medicine, McMaster University, Hamilton, ON, Canada.*

[2]*Global Obesity and Liver Disease Research, Novo Nordisk A/S, Maaloev, Denmark.*

✉*e-mail: gsteinberg@mcmaster.ca*

https://doi.org/10.1038/s41574-021-00529-7

Key points

- Growth differentiation factor 15 (GDF15) is expressed in multiple cell types and can be increased by cellular stressors, including hypoxia, mitochondrial dysfunction, metformin and endurance exercise.
- Increases in GDF15 secretion are mediated through mitochondrial stress and by activation of the integrated stress response pathway as well as, potentially, via AMPK.
- GDF15 reduces the intake of high-fat diets in animal models through binding to glial cell-derived neurotrophic factor family receptor alpha-like (GFRAL) and the recruitment of the receptor tyrosine kinase RET in the hindbrain and this event is required for weight loss and improved glycaemic control.
- Evidence suggests that GDF15 might alleviate non-alcoholic fatty liver disease and non-alcoholic steatohepatitis but the mechanisms mediating the anti-inflammatory effects and whether these are independent of reductions in body weight remain to be determined.
- GDF15 might have cardioprotective effects by reducing atherosclerosis, cardiac hypertrophy and ischaemia–reperfusion injury; however, the mechanisms mediating these effects are still unclear.
- Clinical testing of long-acting analogues of GDF15 is under way and will be important to determine whether the beneficial effects observed in animal models are translated to humans in a safe and efficacious manner.

pharmacotherapies that reduce body mass[21,22], whereas in pregnancy, increases in GDF15 are inversely correlated with maternal BMI[23]. Importantly, these observations are consistent with studies showing that the administration of recombinant GDF15 in rodents[24] and non-human primates[18] reduces food intake and body mass. Therefore, GDF15 could be a potential therapeutic target for obesity and cardiometabolic disease.

In this Review, we discuss the tissue sources of GDF15 as well as its production and secretion. We also highlight what is known about GDF15 signalling. We present findings from preclinical models and human genome-wide association studies (GWAS) that provide evidence that GDF15 might be an effective drug target in cardiometabolic disease. Finally, we summarize the current knowledge on the role of GDF15 in different cardiometabolic diseases.

Tissue expression patterns of GDF15

Tissue source. In lean and healthy mice, *Gdf15* mRNA expression is highest in the kidney, followed by the liver, white adipose tissue (WAT), brown adipose tissue (BAT) and skeletal muscle[25,26]. In mice with obesity, *Gdf15* gene expression is increased in the liver, WAT and BAT to levels similar to those seen in the kidney, where *Gdf15* levels are not changed[18,25]. In contrast to rodents, no difference is observed in *GDF15* mRNA expression in subcutaneous and visceral adipose tissue between lean individuals or people with obesity[16]. This finding suggests that adipose tissue is unlikely to be the primary source for increased GDF15 in human obesity. According to The Blood Atlas, GDF15 is highly expressed in granulocytes and monocytes, where it increases following exposure to inflammatory stimuli[27,28]. Furthermore, activated-immune cells are known to infiltrate the liver in humans with obesity and in animal models of obesity[29]. Thus, immune cells that are found within the liver in obesity could be a potential source for the increased GDF15 observed with obesity. Of note, single-cell RNA sequencing (RNAseq) of human liver biopsy samples from healthy individuals or those with cirrhosis[30] or NASH[31] have shown increased expression of *GDF15* in almost every liver cell type, especially cholangiocytes and endothelial cells. Given the tight correlation between obesity and NAFLD, these studies suggest that the liver might be the tissue contributing to increases in circulating levels of GDF15. Future studies using single-cell RNAseq of various human tissues as well as tissue-specific *Gdf15*-knockout mice will be important to determine the primary cell types contributing to increased GDF15 expression in obesity.

Cellular regulators of GDF15 transcription and GDF15 secretion. In addition to obesity, GDF15 is measured clinically as a potential diagnostic or prognostic biomarker for mitochondrial dysfunction[32], heart failure[33,34] and some cancers[35] such as prostate cancer[36]. Considering the diverse conditions associated with increased GDF15, there are probably numerous cellular regulators of its transcription and secretion. Early reports identified the stress-responsive transcription factors p53 (REFS[37,38]) and early growth response transcription factor (EGR1)[39] as key regulators of GDF15 expression (FIG. 1). The integrated stress response also seems to be of critical importance[25] as several cellular stressors known to activate this pathway, including hypoxia, endoplasmic reticulum stress and amino acid deprivation, have been shown to increase GDF15 expression[25]. Consistent with a vital role for the integrated stress response, *GDF15* transcription requires activating transcription factor 4 (ATF4) and C/EBP homologous protein (CHOP)[10,25,40,41]. As endoplasmic reticulum stress is elevated in multiple tissues during obesity, this mechanism might be important for mediating increases in GDF15; however, further studies are required to examine the relevance of alternative pathways and investigate the most important tissues increasing circulating levels of GDF15 in obesity.

Both ATF4 and CHOP are activated by mitochondrial stress[42] and the mitochondrial unfolded protein response (UPR)[43] — effects that are commonly associated with the development of obesity and insulin resistance. Surprisingly, however, a mouse model of activation of the mitochondrial UPR in muscle due to deletion of *Crif1* (encoding a mitoribosomal subunit) showed improved insulin sensitivity[44]. Subsequent studies identified that this phenotype was associated with marked increases in GDF15 expression in muscle and serum. Importantly, the same phenotype was also observed with other genetic or chemical mitochondrial stressors, leading to the concept that GDF15 is a myomitokine[44]. Similarly, reductions in the function of oxidative phosphorylation subunits within adipose tissue of mice have also been found to increase GDF15 secretion[45]. Although speculative, these findings suggest that increases in GDF15 might be an important mechanism to explain why mice lacking key transcription factors and proteins controlling mitochondrial function often do not develop obesity and insulin resistance as hypothesized[46–49]. Importantly, consistent with studies in rodents with impaired mitochondrial function, GDF15 levels are elevated in humans with age[50,51] as well as in those with mitochondrial diseases[52]. Collectively, these data

Non-alcoholic fatty liver disease
(NAFLD). A spectrum of liver pathology ranging from liver steatosis (>5% lipids) to inflammation and fibrosis, known as non-alcoholic steatohepatitis, that is an important risk factor for type 2 diabetes mellitus, cardiovascular disease, liver cirrhosis and hepatocellular carcinoma.

Integrated stress response
A eukaryotic cellular stress response to restore cellular homeostasis by phosphorylation of eIF2 by four specialized kinases (PERK, GCN2, PKR and HRI), leading to a decrease in global protein synthesis and an increase in the expression of specific genes, including *ATF4*.

Mitochondrial unfolded protein response
(UPR). This cellular stress response is triggered when unfolded or misfolded proteins accumulate in mitochondria beyond the protective capacity of chaperone proteins.

suggest that mitochondrial stress-induced increases in GDF15 might be an important mechanism coordinating mitochondrial capacity with energy intake.

A key protein regulating mitochondrial function is the heterotrimeric enzyme complex AMP-activated protein kinase (AMPK). AMPK is activated by direct allosteric mechanisms involving the β-isoform or indirectly by mitochondrial stressors that increase the AMP or ADP to ATP ratio in a mechanism involving the γ-isoform. A 2021 study showed that activating AMPK in mice using distinct pharmacological activators results in increased liver and serum levels of GDF15 and that this response is blunted in mice lacking the AMPK β1-isoform[53], which is predominant in mouse liver tissue. Interestingly, the effects of AMPK on GDF15 seemed to be UPR independent as GDF15 was similarly increased in wild-type and CHOP-deficient mice. Furthermore, a direct β1-specific allosteric AMPK activator that does not disrupt mitochondrial function increased circulating levels of GDF15 without any apparent effect on the UPR[53]. The mechanism by which AMPK regulates GDF15 expression and secretion remains to be determined. However, considering the relationship between mitochondrial impairments and GDF15 as well as the

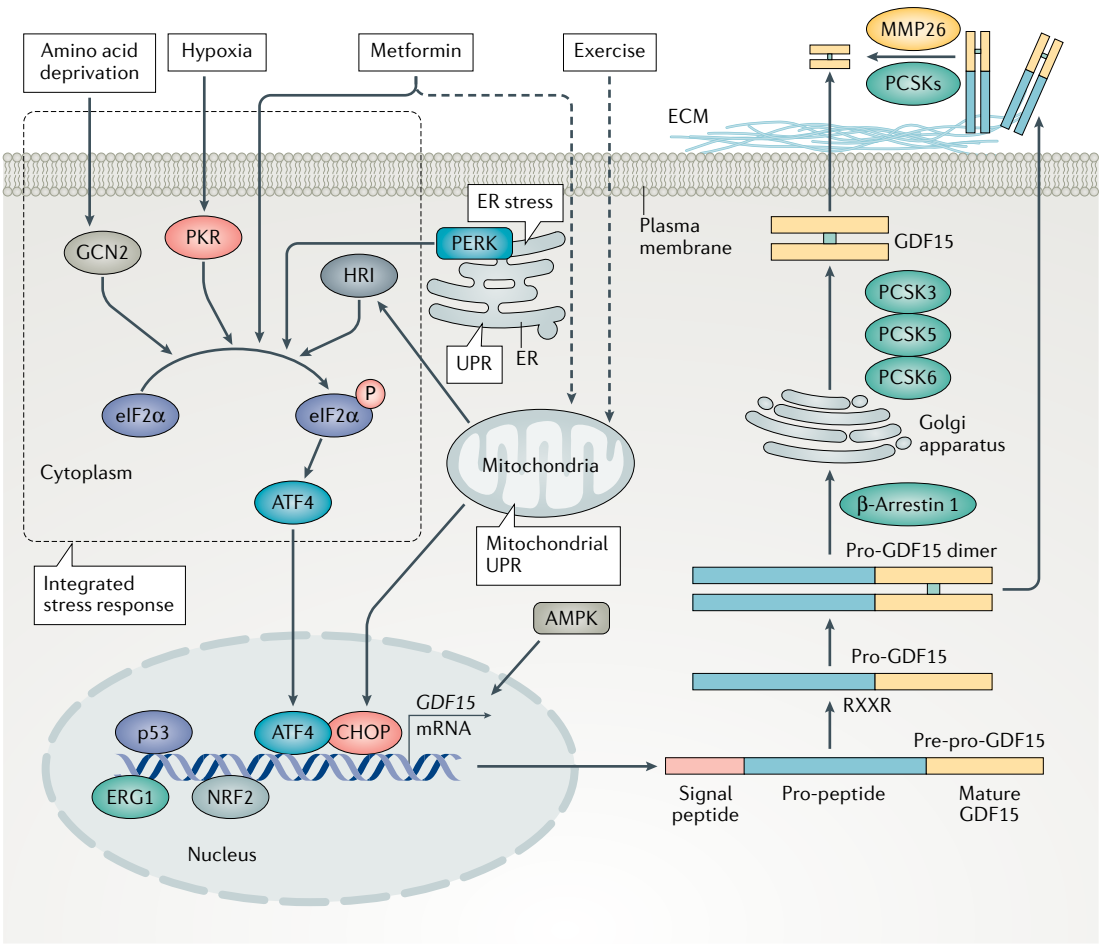

Fig. 1 | **GDF15 regulation, maturation and secretion.** Stress-responsive transcription factors p53 and early growth response transcription factor (EGR1) have been reported as key regulators of growth differentiation factor 15 (GDF15) expression. The integrated stress response (ISR) also seems to be of critical importance to increase GDF15 expression. Stress signals of amino acid deprivation, hypoxia, endoplasmic reticulum (ER) stress and mitochondrial dysfunction activate ISR regulators, GCN2, PKR, PERK and HRI, respectively, which further phosphorylate eIF2α, the core of the ISR. Phosphorylation of eIF2α increases activating transcription factor 4 (ATF4), which forms heterodimers with C/EBP homologous protein (CHOP) that bind to DNA targets to control *GDF15* transcription. Mitochondrial stress and the mitochondrial unfolded protein response (UPR) can also activate this pathway. Activation of AMP-activated protein kinase (AMPK) increases GDF15 expression independently of the UPR. Metformin increases GDF15 expression by a PERK–ATF4–CHOP axis. Metformin and exercise might also increase GDF15 through mechanisms involving mitochondrial function. In the cytoplasm, GDF15 is synthesized as a biologically inactive precursor protein, pre-pro-GDF15, composed of signal peptide, pro-peptide and mature GDF15, which is further cleaved, leaving pro-GDF15. The sixth cysteine in the nine-cysteine domain forms a disulfide bond with a free sixth cysteine from another pro-GDF15 monomer to form a pro-GDF15 homodimer. β-Arrestin 1 facilitates the transport of pro-GDF15 to the Golgi to cleave to mature GDF15 at cleavage site RXXR by proprotein convertase subtilisin–kexin type 3 (PCSK3), PCSK5 and PCSK6. In some types of cells, GDF15 is also secreted as a pro-GDF15 dimer and this pro-domain remains attached to the extracellular matrix (ECM) until it is cleaved by the PCSKs or matrix metalloproteinase 26 (MMP26). Dashed lines indicate processes with unclear mechanisms.

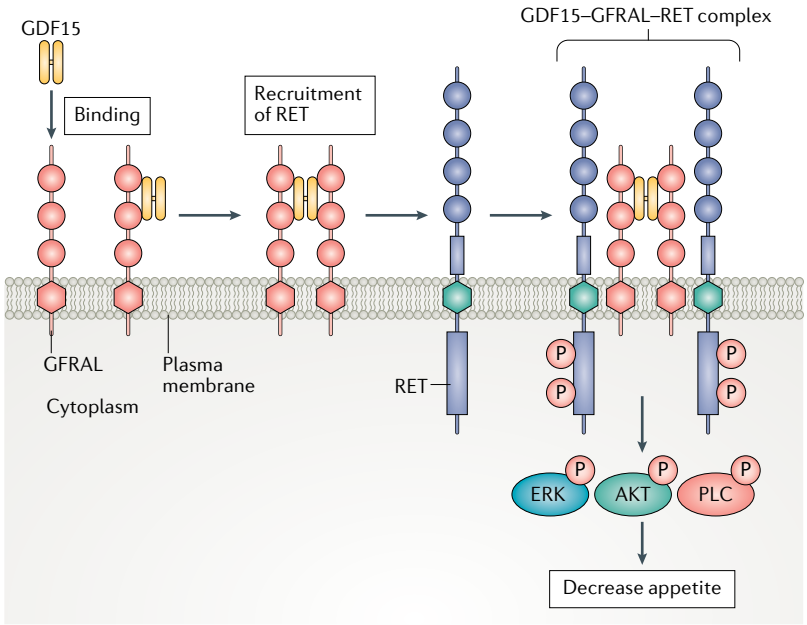

Fig. 2 | **A model of the formation of the GDF15–GFRAL–RET signalling complex.** Growth differentiation factor 15 (GDF15) binds to glial cell-derived neurotrophic factor (GDNF) family receptor alpha-like (GFRAL) and this association leads to the recruitment of RET, which induces the phosphorylation (P) of extracellular signal-related kinase (ERK), RAC-alpha serine–threonine-protein kinase (AKT) and phosphoinositide phospholipase C (PLC). Although RET is expressed in multiple cell types, full-length GFRAL is found predominately in neurons within the area postrema and nucleus tractus solitarus of the hindbrain–brainstem and is required for GDF15-induced suppression of appetite.

importance of AMPK in enhancing mitochondrial function, it is appealing to speculate that AMPK might act as an early response system to stimulate GDF15 before mitochondrial stress develops.

GDF15 maturation and secretion

GDF15 is synthesized as a biologically inactive precursor protein, pre-pro-GDF15 (FIG. 1)[54,55]. The N-terminal signal peptide of pre-pro-GDF15 is important for its trafficking and secretion and is cleaved, leaving pro-GDF15 (~30 kDa). The pro-GDF15 is then cleaved at the C-terminus to result in mature GDF15 (~13 kDa), which forms a homodimer, held together by a disulfide bond, as the major secreted form of GDF15 found in serum[54,55]. In hepatocytes, β-arrestin 1 has been shown to interact with GDF15 and facilitate the transport of pro-GDF15 to the Golgi for cleavage and maturation[56]. Of note, studies in cardiomyocytes under pathological conditions show that proprotein convertase subtilisin–kexin type 3 (PCSK3), PCSK5 and PCSK6 can then cleave pro-GDF15 to GDF15 (REF.[55]).

In some tumour cells, GDF15 is also secreted as a proprotein[57,58] and this pro-domain remains attached to the extracellular matrix until it is cleaved by the PCSKs at the furin-like cleavage site motif RXXR. By contrast, in other cell types, such as placental cytotrophoblasts, this cleavage is mediated by matrix metalloproteinase 26 (REF.[59]). Finally, in some tumour cells, cleavage of GDF15 to a 6-kDa fragment[60] by membrane type 1 pro-matrix metalloproteinase might cleave and thereby inactivate mature GDF15. Collectively, these data suggest that, in addition to transcriptional regulation, a number of post-translational processing events can affect circulating levels of GDF15. However, these mechanisms have only been explored in a fairly limited number of cell and tissue types. Thus, whether the expression of these cleavage enzymes varies across tissues and in various disease states remains incompletely understood. Whether GDF15 fragments might have important biological functions is also unknown.

Circulating levels of GDF15 display a diurnal variation of approximately 10% that is not directly related to meals or caloric consumption[20,25]. Consistent with a minimal response to feeding, the anorexigenic gut peptide glucagon-like peptide 1 (GLP1) does not increase serum levels of GDF15; however, a modest effect of cholecystokinin 8 occurs[20]. Interestingly, 2 weeks of a very low-calorie diet modestly increased serum levels of GDF15 in individuals with obesity but without T2DM[16]. These data suggest that, although circulating levels of GDF15 increase with obesity, levels are not responsive to acute changes in caloric intake. Thus, GDF15 is unlikely to act as a dynamic regulator of appetite under ordinary physiological conditions.

The role of GFRAL in GDF15 signalling

In 2017, manuscripts published by four independent laboratories identified glial cell-derived neurotrophic factor (GDNF) family receptor alpha-like (GFRAL) as a high-affinity receptor for GDF15 (REFS[61–64]). Each paper utilized distinct methodologies, including cell-surface binding microarrays[61–63], fluorescence-associated cell sorting[63] and co-immunoprecipitation assays[64]. Together, they identified that the primary receptor for GDF15 was GFRAL and that GFRAL did not bind with structurally related ligands of the TGFβ or the GDNF family. In addition to GFRAL, GDF15 signalling required the recruitment of a receptor tyrosine kinase, RET (FIG. 2). Three isoforms of RET have been described (RET9, RET43 and RET51), among which the RET51 and RET9 isoforms have distinct signalling activity owing to differential regulation of tyrosine-1062 phosphorylation[65].

GFRAL and RET expression. Consistent with earlier surgical ablation studies that eliminated the appetite-suppressing effects of GDF15 (REF.[66]), GFRAL was shown to be most highly expressed in the area postrema and nucleus tractus solitarus of the hindbrain–brainstem of rodents and primates, with no expression within the hypothalamus[8,61]. By contrast, RET expression is observed in both the hindbrain and the hypothalamus of rodents[62]. In most peripheral tissues, *GFRAL* mRNA is not present, with the exception of low levels in the testis and WAT[63]. Of note, despite the presence of *GFRAL* mRNA in WAT, GFRAL protein content is undetectable by immunohistochemistry in subcutaneous or visceral adipose tissue; therefore, whether a functional receptor is present remains unclear. As with GDF15 expression profiling, adipose tissue infiltrating immune cells might also express GFRAL and processing of tissue for immunohistochemistry could lead to the loss of expression; however, further studies are needed to investigate this possibility. Interestingly, mice have a GFRAL splicing

variant, which lacks both the transmembrane domain and the C3 domain, the latter of which is required for binding with RET[62]. This finding suggests that the splice variant of GFRAL might not have a major influence on GDF15 signalling. However, future studies involving the reconstitution of *Gfral*-knockout mice with the soluble GFRAL receptor are needed to directly evaluate whether it has any functional effects.

GDF15–GFRAL–RET signalling. In a variety of neuroblastoma cell lines, recombinant GDF15 increases the phosphorylation of extracellular signal-related kinase (ERK), RAC-alpha serine–threonine-protein kinase (AKT) and phosphoinositide phospholipase C-γ1 (PLCγ1)[63]. Similarly, in human embryonic kidney cells with stable overexpression of human GFRAL and human RET, GDF15 increased the phosphorylation of RET, ERK1/2 and AKT, whereas the phosphorylation of signal transducer and activator of transcription 3 (STAT3), AMPK, SMAD1, SMAD5 and SMAD9 remained unchanged[62]. With respect to RET isoforms, the RET51–GFRAL complex induces a substantially greater ERK1/2 phosphorylation than RET9–GFRAL or RET43–GFRAL complexes[62]. Of note, these studies[62,63] differ from previous studies examining the effects of GDF15 that found striking similarities with TGFβ signalling[67,68]. This difference has been attributed to TGFβ contamination of many commercial preparations of recombinant GDF15 as TGFβ exerts potent biological actions in the femtomolar range[68]. These data indicate that GDF15 induces activating phosphorylation of ERK1/2, AKT, and PLCγ1 and that GFRAL and RET are required for the effect. Given the very restricted tissue distribution of GFRAL, previous studies examining the effect of purified recombinant GDF15 in cell types lacking GFRAL should be interpreted with caution.

Body mass effects in preclinical models

GDF15 suppresses food intake in animal models and this effect is dependent on the area postrema and nucleus tractus solitarus of the hindbrain–brainstem[66], a region of the brain that is highly enriched in GFRAL (TABLE 1, FIG. 3). The effects of GDF15 on body mass seem to be primarily driven by the suppression of appetite and not by changes in energy expenditure as in mice; matching the reduced caloric intake induced by GDF15 in untreated controls leads to similar degrees of weight loss[62,63,69]. Although the exact brain regions contributing to the reduced food intake are not fully understood (discussed later), studies show that GDF15 might elicit effects through multiple mechanisms, including food preferences, gastric emptying, hedonic hunger, nausea and emesis (FIG. 3).

Food preferences. On a control chow diet, mice with a deficiency in GDF15 or GFRAL have negligible or fairly modest increases in food intake and body mass[64]. However, when challenged with a high-fat diet (HFD), GDF15-null mice have increased food intake, adiposity, insulin resistance and glucose intolerance compared with wild-type mice[61,63,64]. Similarly, acute antagonism of the GDF15–GFRAL axis with either a GDF15 antibody or short hairpin RNA targeting GFRAL also leads to pronounced increases in body weight when mice are fed a HFD[70]. In line with this finding, administered GDF15 has a greater effect on suppressing food intake in rats fed a HFD compared with those fed a high-carbohydrate chow diet[62]. The more pronounced effects of GDF15 on body mass in the context of a HFD might be related to the observations that GDF15 alters taste preferences by specifically reducing appetite for calorie-dense foods[18]. For example, GDF15 treatment decreased the preference for fat intake in wild-type mice but not in mice lacking

Table 1 | **The GDF15–GFRAL axis regulates body weight, glucose control and food intake**

Conditions	Body weight	Glucose control	Food intake or anorexia
GDF15 overexpression	↓Body weight: in nude mice with human prostate cancer[69], in mice fed normal diet[69], in HFD-fed mice[85,101–103]	↑Glucose tolerance and insulin sensitivity in HFD-fed mice[85,101–103]	↓Food intake: in mice fed normal diet[69], in HFD-fed mice[85,101–103]
Recombinant GDF15	↓Body weight: in mice fed a control chow diet[13,69], in obese mouse models: *ob/ob*, *db/db*, KKA^{y} and HFD, in lean and HFD obese rats[62], in obese cynomolgus monkeys[18,63], in shrews[77]	↑Glucose tolerance and insulin responsiveness: in HFD-fed mice[13,18], in obese cynomolgus monkeys[18,63] ↓Plasma levels of insulin in obese cynomolgus monkeys[18,63]	↓Food intake or anorexia: in obese mouse models: *ob/ob*, *db/db*, KKA^{y} and HFD[13,18], in shrews[77]
Gdf15-knockout or anti-GDF15 antibody	↑Body weight: in mice fed a control chow diet[24,70], in HFD-fed mice[70,152]	↑Glucose and insulin levels and ↓glucose tolerance and insulin sensitivity in HFD-fed mice[70,152]	↑Food intake: in mice fed a control chow diet[24,70] or HFD-fed mice[70,152]
Gfral-knockout or anti-GFRAL antibody or adeno-associated virus short hairpin RNA	Eliminates the decrease in body weight by recombinant GDF15: in mice fed a control chow diet[61,64] or HFD[18,62–64]	Eliminates the improved glucose tolerance and insulin sensitivity by recombinant GDF15 in mice fed a control chow diet[61,64]	Eliminates the reduced food intake in mice fed a control chow diet[61,64] in HFD-induced obese mice[18,62–64]
	Conflicting data: ↑body weight in HFD-fed mice[61,63,64,70] or no change in body weight in HFD-fed mice[62]	↓Glucose tolerance and insulin concentrations in HFD-fed mice but not in mice fed a control chow diet[61,63,64], ↑insulin concentrations[63]	Conflicting data: ↑food intake in mice fed with HFD but not a control chow diet[61,63,64]; oppositely, no influence on food intake in HFD-fed mice[62]
Metformin	↓Body mass and adiposity in WT but not GDF15 KO HFD-fed mice[10,41]	↑Glucose tolerance, insulin sensitivity, ↓fasting insulin in WT but not *Gdf15*-knockout HFD-fed mice[10,41]	↓Food intake in WT but not *Gdf15*-knockout HFD-fed mice[10,41]

↑, increase; ↓, decrease; GDF15, growth differentiation factor 15; GFRAL, glial cell-derived neurotrophic factor family receptor alpha-like; HFD, high-fat diet; WT, wild-type.

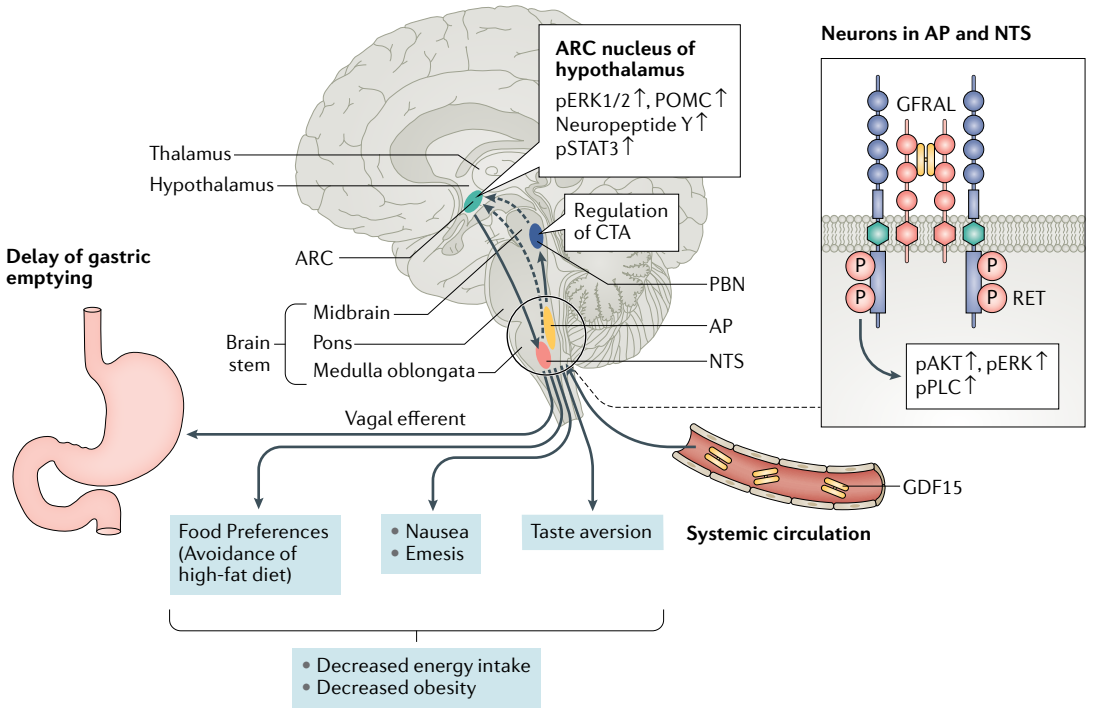

Fig. 3 | **Proposed mechanisms by which GDF15 suppresses energy intake and obesity.** Activation of growth differentiation factor 15 (GDF15)–glial cell-derived neurotrophic factor family receptor alpha-like (GFRAL)–RET signalling in the area postrema (AP) and nucleus tractus solitarus (NTS) of the brainstem projects to the parabrachial nucleus (PBN) (which might induce conditioned taste aversion (CTA)) and to arcuate (ARC) hypothalamic nuclei, which modulate vagal sympathetic nervous system activity (vagal efferent), delaying gastric emptying. Physiological levels of GDF15 also affect food preferences, limiting the consumption of diets high in fat. At high doses, GDF15 might also induce nausea and emesis in some species. GDF15-induced reductions in appetite and body mass do not require leptin, glucagon-like peptide 1 (GLP1) or the melanocortin 4 receptor, although there might be some overlap with these signalling pathways (for example, extracellular signal-related kinases 1/2 (ERK1/2), STAT3, pro-opiomelanocortin (POMC), neuropeptide Y) within the ARC nucleus of the hypothalamus through mechanisms that are currently not understood.

GFRAL[18,71]. Of note, these findings are quite different from mice lacking leptin (*ob/ob*) or leptin receptor signalling (*db/db*), which are hyperphagic and develop obesity when fed either a high-carbohydrate chow diet or a HFD. Why a hormone that is increased in response to cellular metabolic stress in multiple tissues is more efficacious when animals are fed a HFD remains unclear. Futures studies investigating mechanisms linking GDF15 with fatty acid metabolism and appetite control are warranted.

Interactions with leptin and GLP1. The effects of GDF15 on food intake are independent of other hunger or satiety hormones such as leptin[69] and GLP1 (REF.[63]). For example, leptin suppresses food intake normally in *Gfral*-knockout mice[62] and GDF15 induces weight loss in obese leptin-deficient *ob/ob* mice[69]. Similarly, a deficiency in melanocortin 4 receptor[61] or the GLP1 receptor[71] does not reduce the anorexic effects of GDF15. Finally, synergistic effects occur on food intake and weight loss, when mice are treated with both GDF15 and the GLP1 receptor agonist liraglutide, indicative of distinct mechanisms of action for these molecules[71]. However, it should be noted that some overlap might exist between GDF15 signalling and classic appetite control pathways. For example, similar to leptin, the injection of mice with recombinant GDF15 induces the phosphorylation of ERK1/2 and STAT3 within the arcuate nucleus of the hypothalamus (ARC, a major centre for appetite control). Furthermore, this effect is associated with increased expression of appetite-suppressing neuropeptides, neuropeptide Y (NPY) and pro-opiomelanocortin (POMC)[69,72]. These canonical neurons and regions of appetite regulation, including NPY–POMC neurons in the ARC, are homeostatic systems where circulating signals (like ghrelin and leptin) inform the brain of energy stores, so food intake can be adjusted to match expenditure[72]. Thus, POMC and NPY might potentially be involved in GDF15-regulated food intake[73,74]. In addition to the hypothalamus, another report has indicated that GDF15 induced anorexia in mice probably through activating cholecystokinin neurons in the area postrema and nucleus tractus solitarus[75]. Future studies exploring other possible interactions between classic appetite-regulating hormones and dietary preferences, including the orexigenic hormone ghrelin, will be important to further understand the mechanisms by which the GDF15–GFRAL signalling axis supresses appetite.

Gastric emptying. In addition to regulating appetite control centres in the brain, the treatment of mice with GDF15 delays gastric emptying[18]. Vagal efferent and afferent signals have important roles in postprandial

gastric emptying[76]. Of note, the GDF15-induced delay of gastric emptying disappears in mice after bilateral subdiaphragmatic vagotomy, suggesting that GDF15 might act through a vagal-mediated mechanism[18]. However, GDF15-inhibited food intake is not impaired in rats when the selective vagal de-afferentiation technique is used to block the vagal afferent[62], which suggests that the GDF15-mediated suppression of food intake is not associated with the vagal afferent but only with the vagal efferent.

Nausea, emesis and taste aversion. GDF15 causes emesis in musk shrews and induces behaviours indicative of nausea (for example, anorexia and pica) and decreases hedonic responses in non-emetic species (mice and rats). These findings suggest that GDF15-induced decreases in food intake involve nausea and emesis[77]. Of note, serotonin plays a key role in emesis[78] and the serotonin receptor 3 antagonist, ondansetron, is a widely used anti-emetic[79] that only partially attenuates GDF15-induced anorexia in mice[77]. However, as ondansetron also reduces gastric emptying, this effect might potentially also be important for suppressing the GDF15 response[80]. Future studies examining whether GDF15-mediated reductions in food intake involve serotonin-induced emesis or gastric emptying are required.

In addition to emesis, other studies have suggested that GDF15 might suppress appetite by inducing a conditioned taste aversion response[25,81,82] — a finding consistent with observations that GDF15 activates neurons and induces cFOS within the parabrachial nucleus and central amygdala[8,25,61,82]. Under pathological conditions, such as stress, trauma and illness, these neurocircuits can override energy homeostasis and reduce food intake irrespective of how much body weight is lost[83]. In addition, with repeated stimulation of these circuits, animals remain anorexic even when threatened with death from starvation[84]. These findings might have particular relevance for cancer cachexia or treatment with noxious chemotherapeutics. For example, in *Gfral*-knockout mice, cisplatin still increases levels of GDF15 but does not induce weight loss or adipose tissue loss or reduce food consumption as observed in wild-type mice[61].

Secondary adaptations associated with energy expenditure. Although pair-feeding experiments have indicated that GDF15-induced weight loss is primarily due to reduced food intake[62,63,69], chronically (for example, 16 weeks) elevated levels of GDF15 might exert some secondary adaptations related to energy expenditure[85]. For example, some studies showed that elevated circulating levels of GDF15 increased the mRNA expression of uncoupling protein 1 (*Ucp1*) in inguinal WAT[44] and BAT[85] of mice independently of changes in food intake; however, this effect was not seen in other studies[13]. A 2020 study revealed that one potential mechanism for GDF15 to mediate the browning of WAT and subsequent increases in energy expenditure might involve GDF15 recruiting or activating anti-inflammatory M2 macrophages[45]. Other studies have suggested that GDF15 might enhance energy expenditure due to a switch from carbohydrate to lipid oxidation[44,61]. This finding is consistent with studies in tumour-bearing mice, which showed that GDF15 increased lipid oxidation and decreased glucose oxidation through increases in lipolysis that were mediated by adipose triglyceride lipase (ATGL)[86]. To determine that the observed effects are not secondary to reduced food intake and carbohydrate availability, future studies are needed in which energy expenditure is measured before appreciable changes in body mass and/or with appropriate pair-feeding controls. Another important consideration when assessing energy expenditure in rodents is housing temperature. All studies to date have been conducted in rodents housed at room temperature (21 °C), leading to the upregulation of adaptive thermogenesis, which can mask subtle differences in energy expenditure mediated through diet-induced thermogenesis. Therefore, it will be important for future studies assessing energy balance to be conducted in rodents housed at thermoneutrality (28–30 °C) as this condition might more accurately predict whether effects will be translatable to humans. For example, weight loss elicited by GLP1 receptor agonists increases adipose tissue browning and energy expenditure in rodents housed at room temperature[87,88] but this effect is not observed when mice are housed at thermoneutrality[89] or in humans[90].

Collectively, these data suggest that, much like GLP1 (REF.[91]), GDF15 reduces appetite via multiple distinct mechanisms, which contribute to its beneficial effects on reducing body mass. Future studies are needed to determine at what doses these effects are seen and which ones translate to humans.

Evidence from GWAS

As no clinical trials testing the effects of GDF15 in humans have been disclosed, whether the association between plasma levels of GDF15 and body weight in humans is causative or correlative remains unclear. One strategy to shed more light on this knowledge gap is to investigate if genetic variation in and around *GDF15* and *GFRAL* is associated with obesity or obesity-related phenotypes. Notably, the genomic interval around *GDF15* is linked by GWAS to a wide array of cardiometabolic traits, including coronary artery disease, cholesterol, waist-to-hip ratio and BMI (Common Metabolic Diseases Knowledge Portal). Although the interval contains multiple interesting candidate genes, such as the neighbouring *PGPEP1*, several common variants suggest a causal contribution by *GDF15* and *GFRAL* specifically to BMI (TABLE 2).

The Genetic Investigation of ANthropometric Traits (GIANT)-UK Biobank meta-analysis of almost 700,000 individuals[92,93] demonstrated that GDF15 intronic variant rs10424912 and downstream variant rs16982345 are associated with BMI. Expression quantitative trait loci (eQTL) and protein quantitative trait loci analyses from an eQTLGen project pre-print paper[94] and a paper by Sun et al.[95] show that the rs16982345 haplotype affects the levels of GDF15 in the blood. Further eQTL analyses from the GTEx project demonstrate that the rs1042912 haplotype affects the expression of

Cancer cachexia
This state is characterized by reductions in appetite and increases in energy expenditure, which leads to involuntary loss of adipose and lean mass that is associated with poor quality of life and reduced survival.

Table 2 | **The possible effect of *GDF15* or *GFRAL* variants on obesity based on GWAS studies**

Gene	dbSNP ID	Type	Trait	Dataset	*P* value	Effect size	MAF	GDF15 eQTL tissue
GDF15	rs16982345	Downstream	BMI	GIANT-UKBB MA	4.35×10^{-9}[a]	0.012	0.25	Blood
GDF15	rs10424912	Intronic	BMI	GIANT-UKBB MA	3.72×10^{-8}[a]	−0.011	0.42	Whole blood; tibial artery
GDF15	rs1227732	Intronic	WHR-adjusted BMI	GIANT-UKBB MA	3.17×10^{-7}	0.013	0.19	Whole blood
GDF15	rs62122429	Intronic	WHR-adjusted BMI	GIANT-UKBB MA	3.37×10^{-7}	0.013	0.19	Whole blood
GDF15	rs1227733	3′-UTR	WHR-adjusted BMI	GIANT-UKBB MA	3.83×10^{-7}	0.013	0.19	Whole blood
GFRAL	rs12199003	Missense	BMI	GIANT Exome Chip	3.40×10^{-9}[a]	0.013	0.34	NA

dbSNP ID, single nucleotide polymorphism database identification number; eQTL, expression quantitative trait loci; GDF15, growth differentiation factor 15; GIANT-UKBB MA, Genetic Investigation of ANthropometric Traits-UK Biobank meta-analysis; GWAS, genome-wide association study; MAF, minor allele frequency; WHR, waist-to-hip ratio; BMI, body mass index; NA, not available. [a]Genome-wide significance.

GDF15 in whole blood and tibial artery[96]. Interestingly, three additional non-coding variants within the *GDF15* gene belonging to a common haplotype are associated with waist-to-hip ratio-adjusted BMI without reaching genome-wide significance[97]. This haplotype also demonstrated an eQTL effect on GDF15 in whole blood[96]. A parallel study of coding variants in over 718,000 individuals demonstrated that the *GFRAL* missense variant rs12199003 (p.Arg33Cys) is associated with BMI[98]. This variant is in the C1 domain of GFRAL[99] and although this residue is not known to be directly involved in the interaction with GDF15 (REF.[61]), it is still required for GDF15 binding of GFRAL[62] and thus might still influence binding with GDF15. Despite several lines of evidence from GWAS linking GDF15 and GFRAL with BMI, a 2019 study[100] applying Mendelian randomization methods found no causal relationship between GDF15 and BMI or other obesity-related traits besides estimated bone mineral density. Taken together, additional studies are seemingly needed to examine whether physiological levels of GDF15 regulate adiposity and cardiometabolic traits. However, it must be considered that such studies do not directly evaluate the potential of supraphysiological levels of GDF15 or direct targeting of GFRAL in the treatment of obesity and cardiometabolic disease.

GDF15 and T2DM

Obesity is a major risk factor for the development of T2DM, making it difficult to detangle the connections between GDF15 and glucose homeostasis. The treatment of rodents[13,18,85,101–103], shrews[77] and monkeys[18,63] with GDF15 decreases body weight and reduces blood levels of glucose (TABLE 1). Importantly, pair-feeding experiments in rodents have indicated that calorically matching food consumption of control-treated mice to GDF15-treated mice leads to similar reductions in body mass but also in blood levels of glucose, which suggests that the regulation of GDF15 on glucose control is primarily due to reduced food intake and body mass[62,63,69]. Similarly, some small epidemiological and retrospective analyses of clinical trials have found connections between GDF15, blood levels of glucose and the development of T2DM[16,104,105]; however, this association has not been observed in larger trials after correction for covariates, including body mass, age and sex[106,107]. Although speculative, one reason why initial studies might have reported increases in GDF15 with T2DM[16,104,105] might have been the common use of metformin in people with T2DM, which, as discussed in detail later, increases circulating levels of GDF15 (REF.[21]). However, the regulation of insulin secretion and inflammation by GDF15 might be of importance in the regulation of blood levels of glucose (described later) and requires further study.

Insulin secretion. A 2020 study has indicated that GDF15 might decrease blood levels of glucose independently of changes in body mass through the direct regulation of insulin secretion. Specifically, GDF15 levels were decreased by 90% in islets of patients with type 1 diabetes mellitus but not in those with T2DM compared with matched controls[108]. GDF15 expression was also reduced in islets from insulin-deficient non-obese diabetic mice and in mouse and human islet cell lines treated with IL-1β and IFNγ[108]. The authors proposed that GDF15 inhibited insulitis and oxidative stress; however, whether GDF15 regulates β-cell apoptosis remains to be investigated as GFRAL was not detected in the islets[63].

GDF15 and inflammation. Chronic low-grade inflammation is an important contributing factor to the development of insulin resistance; therefore, one potential mechanism linking GDF15 with glucose homeostasis involves reducing inflammation[70].

In humans, GTEx RNAseq data analysis indicate that *GDF15* gene expression positively correlates with the expression of inflammation-related genes in the liver, adipose tissue and skeletal muscle from humans[51]. Similarly, culturing of senescent $CD8^+$ T cells from people with pre-diabetes with hepatocyte-like HepG2 cells also increases GDF15 expression[109]. Other studies have found that an SNP in *GDF15* associated with increased expression is linked to a decreased total number of leucocytes, innate immune cells, and myeloid white cells and an increased concentration of lymphocytes and monocytes in the blood[51]. These data suggest that, in humans, there might be linkages between GDF15 and low-grade chronic inflammation. However, the mechanisms mediating these effects are currently unclear as the Human Protein Atlas suggests that GFRAL expression is extremely low in most immune cell types, including monocytes and T cells.

In obese mice, genetic overexpression of GDF15 in all tissues or administration of recombinant GDF15 reduces markers of inflammation in serum[13] and WAT[24,103]. Conversely, the treatment of obese mice fed a HFD with a monoclonal antibody that inhibits GDF15

leads to greater adiposity and markers of adipose tissue inflammation (*Tnf*, *Il6*, *Mcp1*, *Cd68*, *F480*)[70]. However, interpreting whether GDF15 exerts anti-inflammatory effects on adipose tissue macrophages is complicated in these studies as alterations might have been secondary to differences in body weight and adipose tissue cell size.

Some studies have revealed a potential anti-inflammatory effect of GDF15 on macrophages independently of changes in obesity and/or total adipose tissue volume. For example, when mice are fed a HFD and are depleted of tissue-resident macrophages using clodronate, reconstitution of the bone marrow with bone marrow from GDF15-deficient mice has a relatively modest effect on increasing body mass but results in a dramatic increase in pro-inflammatory macrophages ($CD11c^+CD206^-$) within epididymal WAT[110]. Subsequently, in the same study, it was shown that GDF15 deficiency blocked the anti-inflammatory effects of IL-4 (REF.[110]). Similarly, in a separate study[111], HFD-fed mice deficient in GDF15 were also resistant to the anti-inflammatory effects of IL-13. Importantly, in both studies, mice lacking GDF15 had greater glucose intolerance, supporting an important potential link between the anti-inflammatory effects of GDF15, resolution of insulin resistance and improved glycaemic control in T2DM. However, it is critically important to understand the mechanism of how GDF15 might exert anti-inflammatory effects independently of changes in adiposity. In isolated bone marrow-derived macrophages[103] or the human macrophage THP1 cell line[31], treatment with recombinant GDF15 has been found to reduce the induction of pro-inflammatory cytokines by lipopolysaccharide or fatty acids. Additionally, in mouse bone marrow-derived macrophages, this effect was linked to increases in oxidative metabolism[103]. However, the interpretation of these findings is complicated as GFRAL is not expressed in mouse bone marrow-derived macrophages or THP1 cells (Human Protein Atlas), which suggests that there might be alternative GDF15 receptors in immune cells or that recombinant GDF15 might have been contaminated with TGFβ as has been described previously[68]. Therefore, the weight-independent mechanisms mediating the potential anti-inflammatory effects in macrophages remains unresolved.

Collectively, these data suggest that GDF15 might reduce blood levels of glucose by promoting insulin secretion and reducing low-grade chronic inflammation. However, whether this effect is secondary to its effects on adiposity or is via direct anti-inflammatory effects remains to be determined[15]. Furthermore, how GDF15 might reduce inflammation in immune cells remains unclear as immune cells have low expression of GFRAL as described earlier. Studies are now needed to examine whether immune modulation by GDF15 is mediated through direct effects on immune cells or via the central nervous system. This information will be important for evaluating whether GDF15 exerts positive effects on insulin sensitivity and insulin secretion independently of reductions in body mass and/or adiposity. Finally, examining the effects of acute increases of GDF15 levels on tissue-specific insulin secretion and insulin sensitivity using hyperinsulinaemic–euglycaemic clamps, independent of changes in food intake and body mass, will be important to determine whether GDF15 has direct effects on the regulation of blood glucose levels in diabetes mellitus.

Metformin and GDF15

Metformin is the most commonly prescribed medication for the treatment of T2DM due to its glucose-lowering effects, which are linked to drug action in the gastrointestinal tract and liver, where drug concentrations are the highest[112]. Interestingly, metformin has been shown to exert a large array of positive effects across multiple tissues of the body and in many conditions, including cancers, CVD and ageing[113]. These beneficial effects led to the hypothesis that metformin might induce the expression of an endocrine factor that communicates beneficial effects across the body[113]. Initial studies examined this hypothesis through analysis of serum samples from over 8,000 people with dysglycaemia, of which 2,317 were taking metformin; out of 235 proteins analysed, GDF15 was increased more than any other protein by metformin treatment[21]. Importantly, this effect was independent of age, sex, HbA_{1c}, and BMI and was not observed with other glucose-lowering therapies, which suggests that the effect was specific to metformin and not due to changes in blood levels of glucose. Subsequent studies in a randomized clinical trial involving metformin confirmed these observations[114]. However, the relevant tissue and whether GDF15 was important for mediating any beneficial effects of metformin remained unknown.

In mice, metformin acutely increased the mRNA and secretion of GDF15 from hepatocytes[41] and the small intestine[10]. Further investigations in mice fed a HFD found that metformin suppressed appetite when delivered in drinking water[10,41] and decreased body mass[10,41] — effects that were eliminated in *Gdf15*-knockout mice[10,41]. Genetic deletion of GFRAL or the use of a GDF15 antibody also prevented the metformin-induced reduction in body weight[10]. This relationship between metformin, GDF15 and body mass were independent of GLP1 and did not seem to involve increases in energy expenditure[41]. Interestingly, the effects of metformin on appetite control were only dependent on GDF15 if the mice were fed a HFD[41], adding further credence to the concept that GDF15 might alter food preferences away from diets high in lipids. Surprisingly, the beneficial effects of 10 weeks of metformin exposure on improving glucose tolerance and lowering fasting insulin were eliminated in *Gdf15*-knockout mice fed a HFD[41]; however, this dependence of GDF15 on metformin-associated metabolic improvements was not observed in another study after just 11 days of treatment[10]. These data suggest that, over shorter durations, metformin improves glucose homeostasis independently of GDF15 but, over longer durations in which differences in body mass are more notable, GDF15 is an important factor for improving insulin sensitivity.

Interestingly, metformin-induced increases in GDF15 secretion were almost completely ablated in hepatocytes of *Chop*-knockout mice or after small interfering

RNA-mediated *ATF4* knockdown (FIG. 1), whereas AMPK did not seem to be essential[41]. Of note, human data show that serum concentrations of GDF15 are correlated with weight loss in people taking metformin[10,41]. Collectively, these data indicate that metformin stimulates the release of GDF15 through the UPR and this effect is important for weight loss and improvements in glycaemic control. Whether increases in GDF15 are important for other beneficial effects of metformin, such as CVD and certain cancers, remains to be determined.

Exercise and GDF15

Similar to metformin, exercise has been shown to lower blood levels of glucose and reduce the incidence of T2DM in people with obesity and insulin resistance[115]. Furthermore, aerobic exercise (1 hour at 67% of VO_2 max) increases blood levels of GDF15 (~34%) in healthy individuals (FIG. 1)[116]. Longer and more extreme exercise (an ultramarathon) increases GDF15 levels even further (~fourfold)[117]. In addition, individuals with obesity who participated in a 12-week aerobic exercise intervention (1 hour per day, 5 days per week with isocaloric diet) had modest increases in plasma levels of GDF15 (REF.[118]).

In line with these data, moderate-intensity endurance exercise or high-intensity sprinting in a range of diverse human populations, including lean people, individuals with obesity, older adults, sedentary people and trained individuals, increase GDF15 (REFS[119,120]). By contrast, in heart transplant recipients, neither high-intensity interval training nor moderate-intensity continuous training changed plasma levels of GDF15, potentially due to already elevated levels of the protein in this population[121].

The tissue source of GDF15 during exercise remains to be determined. In mice, *Gdf15* mRNA is increased twofold in liver, heart and soleus muscle immediately after exercise, which suggests that these tissues contribute to the increased circulating levels of GDF15 (REF.[120]). However, in healthy individuals, venous and arterial concentrations of GDF15 across the exercising leg are similar, which indicates that skeletal muscle is unlikely to be the primary source for increases in circulating levels of GDF15 (REF.[116]). Given the much higher concentration of GDF15 in the liver compared with skeletal muscle[120] and the severe energetic stress that the liver undergoes during aerobic exercise[122,123], it might be speculated that the primary source of GDF15 during aerobic exercise is the liver; however, future studies directly testing this hypothesis are required.

So, what might the function of exercise-induced increases in GDF15 be? Mice dosed acutely with GDF15 perform less voluntary wheel running than mice treated with vehicle control but there is no effect on forced treadmill running[120], which suggests that pharmacological levels of GDF15 decrease the motivation to exercise but not the ability to do so. However, the physiological induction of GDF15 in response to exercise does not seem to modulate exercise behaviour or post-exercise energy intake as a deletion of GFRAL did not affect exercise performance or intake of a chow diet after exercise in mice[120]. Thus, increases in GDF15 during exercise do not seem to be responsible for reducing exercise performance, the desire to exercise or food intake. The reason why high levels of GDF15 decreased voluntary exercise in mice is unknown but might be related to nausea and/or the presence of yet unidentified central nervous system circuits critical for the regulation of exercise behaviour. Although the relationship between exercise and GDF15 is just beginning to be explored, one study has indicated that GDF15 could play a role in increasing adipose tissue lipolysis[119]. It is also interesting to speculate on another possibility that increases in GDF15 during exercise might be important for promoting glycogen resynthesis after exercise by altering food preferences away from high-fat foods, which, by default, would lead to the increased consumption of carbohydrates. Lastly, whether GDF15 mediates any of the numerous benefits of exercise on multiple disease conditions, including T2DM, CVD and cancer, will be interesting to determine.

NAFLD and NASH

The incidence of NAFLD mirrors the prevalence of obesity and T2DM. In mice, GDF15 expression is increased in the liver of obese mice, which have hepatic steatosis, compared with lean controls[40]. Liver GDF15 levels are also increased in two different dietary mouse models of NASH: the methionine–choline-deficient (MCD) diet model and the amylin liver NASH diet model. Of note, NASH develops independently of obesity in MCD diet-fed mice, which suggests that GDF15 increases are not due to adiposity[124]. Moreover, GDF15 mRNA was upregulated in the liver but not in skeletal muscle, BAT or WAT in the MCD model, thereby suggesting that systemic increases in GDF15 might originate from the liver in this model[124]. In an in vitro model of NASH, induced by media completely deficient in methionine and choline, increases in GDF15 were observed in hepatocytes but not in hepatic stellate cells or in Kupffer cell lines, which suggests that hepatocytes are the primary source of elevated GDF15 (REFS[40,124]).

In humans, circulating levels of GDF15 are increased sequentially with disease progression, being highest in advanced fibrosis, followed by NASH and NAFLD[125]. Among patients with NAFLD, the highest quartile of circulating GDF15 was positively associated with the risk of advanced fibrosis and liver stiffness after adjustment for BMI and other factors. These findings suggest that GDF15 could be a prognostic biomarker to predict advanced fibrosis in NAFLD[125]. Another very comprehensive study that analysed >400 individual liver biopsy samples from patients with NAFLD or NASH using a combination of unbiased RNAseq and proteomics data found that the serum level of GDF15 is increased during the progression of NAFLD–NASH and is positively associated with worsening fibrosis stage, steatosis, activity, inflammation scores and activity of steatohepatitis[31]. Based on these studies, levels of GDF15 in serum and liver are increased in NAFLD and NASH and hepatocytes might be the primary source of this increase; however, further studies evaluating this hypothesis are required.

In HFD-fed mice, recombinant GDF15 or GDF15 overexpression reduces body mass and hepatic

steatohepatitis as well as the infiltration of inflammatory cells[13,85]. Furthermore, GDF15 overexpression also reduced the expression of inflammatory and fibrotic genes without changing body weight in the MCD diet-fed model, which suggests weight-independent effects[124]. Similar effects on NASH were observed in mice treated with a liver-directed adenovirus expressing GDF15 (REF.[40]). Consistent with this effect, inhibiting GDF15 increased liver mass and lipid deposition as well as body weight in HFD-fed mice[70,124] and aggravated MCD diet-induced NASH without changing body weight[124]. Mechanistically, these effects of GDF15 have been shown to involve the increased expression of β-oxidation-related genes within the liver and in hepatocytes treated with GDF15 (REFS[40,124]), which adds further evidence that effects might be independent of weight loss[124]. In addition, GDF15 suppressed the expression of fibrogenic genes, such as *Tgfb1*, *Col1a1*, *Timp1* and *Acta2*, in hepatic stellate cells from mice. In mice with alcoholic liver disease and CCl_4-induced liver fibrosis, GDF15 deficiency also promotes hepatic lipid accumulation and immune cell infiltration, leading to increases in serum levels of tumour necrosis factor and IL-6 and the liver expression of pro-inflammatory cytokines; these effects were reversed with recombinant GDF15 (REF.[126]).

Taken together, these data suggest that GDF15 could be a promising target for the treatment of NAFLD or NASH; however, as GFRAL is not expressed in hepatocytes[62], further studies are needed to determine how GDF15 might exert beneficial effects on NASH, independently of reductions in appetite and body mass (FIG. 4). One possible hypothesis is that as GDF15 increases hepatic triglyceride export via sympathetic outflow and adrenergic signalling[127], this peripheral sympathetic axis could conceivably contribute to the positive effect. Further mechanistic studies are required to understand how GDF15 might exert positive effects in NASH and whether this mechanism could be of importance in humans.

Cardiovascular disease

Increased plasma levels of GDF15 are an established biomarker for cardiac hypertrophy, heart failure, atherosclerosis and endothelial dysfunction[128]. GDF15 is weakly expressed in healthy human cardiovascular tissues, whereas its expression is increased in cardiomyocytes and cardiac tissue in response to cardiovascular injury induced by pressure overload, heart failure, ischaemia–reperfusion injury and atherosclerosis[33,128–130]. The exact cell types contributing to this increase in GDF15 are not known but could be heart-resident macrophages[128]. Importantly, as has been reviewed extensively, GDF15 is a biomarker associated with unfavourable prognosis in CVD[33,128,131]; therefore, the focus of the following sections will be on the potential therapeutic application of GDF15 in CVD (FIG. 4).

Atherosclerosis. Atherosclerosis is caused by the build-up of lipid-laden foam cells in the artery walls, which triggers the further development of complex plaques with necrotic cores, cholesterol crystals and fibrous collagen-rich caps[132,133]. Atherosclerotic plaque size and stability are important factors as they contribute to the occlusion of arteries and to the risk of rupture and thrombotic events that cause heart attacks and strokes[134]. Of note, GDF15 expression is increased in cultured peritoneal macrophages that are stimulated with oxidized LDL[135]. Moreover, akin to humans, GDF15 expression is upregulated in mice as atherosclerosis progresses and this increased expression is primarily colocalized within macrophages of the plaque[136]. Thus, increases in GDF15 are associated with the development of atherosclerosis. However, much like studies with obesity, this increase does not necessarily mean that GDF15 promotes atherogenesis. The studies described in this section are contradictory, with some showing that GDF15 might promote atherogenesis while others indicate that it could be protective.

In support of GDF15 promoting atherogenesis, mice deficient for both ApoE and GDF15 have reduced lumen stenosis and decreased ^{18}F-fluorodeoxyglucose uptake in the aortic arch compared with ApoE-expressing controls, suggestive of decreased macrophage inflammation in atherosclerotic plaques[135]. The authors attribute this reduced inflammation in ApoE GDF15-deficient mice to reduced induction of IL-6 within peritoneal macrophages by oxidized LDL[135]. Similarly, haematopoietic GDF15 deficiency in *LDLR*-knockout mice reduced initial lesion formation but increased collagen in later lesions, suggesting that GDF15 promotes early atherogenesis but might improve plaque stability[136]. Consistent with this idea, GDF15 deficiency in mice lacking LDLR decreased necrotic core formation and macrophage infiltration in plaques, possibly via reduced C-C chemokine receptor type 2 expression, without affecting total lesion size[136]. These data suggest that, in both ApoE and LDLR mouse models of atherosclerosis, GDF15 might promote the development of atherogenesis.

By contrast, some studies suggest that GDF15 might exert protective effects in atherosclerosis. For example, the transplantation of bone marrow from GDF15-deficient mice to *Ldlr*-knockout mice did not influence lesion size but increased macrophage accumulation. This effect probably occurred via the inhibition of intercellular adhesion molecule 1 expression and increased atherosclerotic plaque destabilization such as by the thinning of fibrous caps[137]. In addition, GDF15 overexpression decreases atherosclerotic lesions in *Apoe*-knockout mice fed a HFD[138]. The reasons for these contradictory findings of GDF15 on atherosclerosis are unclear but might be due to a variety of parameters, including mouse genetic backgrounds and housing temperatures, which can both have large effects on atherosclerosis development[139,140].

The recruitment of monocytes to the arterial wall and macrophage polarization play important roles in the initiation and development of atherosclerotic plaques[133]. At the initiation of atherosclerosis, the recruitment of monocytes under arterial endothelium facilitates the clearing of accumulated lipid from the sub-endothelial space. However, excess cholesterol accumulation in macrophages leads them to transform into foam cells, which contribute to atherosclerotic development[133]. As discussed in the T2DM section earlier, GDF15 inhibits the

Foam cells
Macrophages or vascular smooth muscle cells with a foamy appearance, which are over-laden with lipids and are a key cell type contributing to the development of atherosclerotic cardiovascular disease (coronary artery disease).

recruitment of neutrophils, which leads to the inhibition of monocyte recruitment[141]. Therefore, GDF15 deficiency might promote monocyte recruitment into the sub-endothelial space in order to remove excess lipids, which could limit initial lesion formation[136]. However, in the late phase of atherosclerosis, GDF15 might induce beneficial effects on atherosclerosis by inhibiting monocyte recruitment and macrophage activation[141]. Further

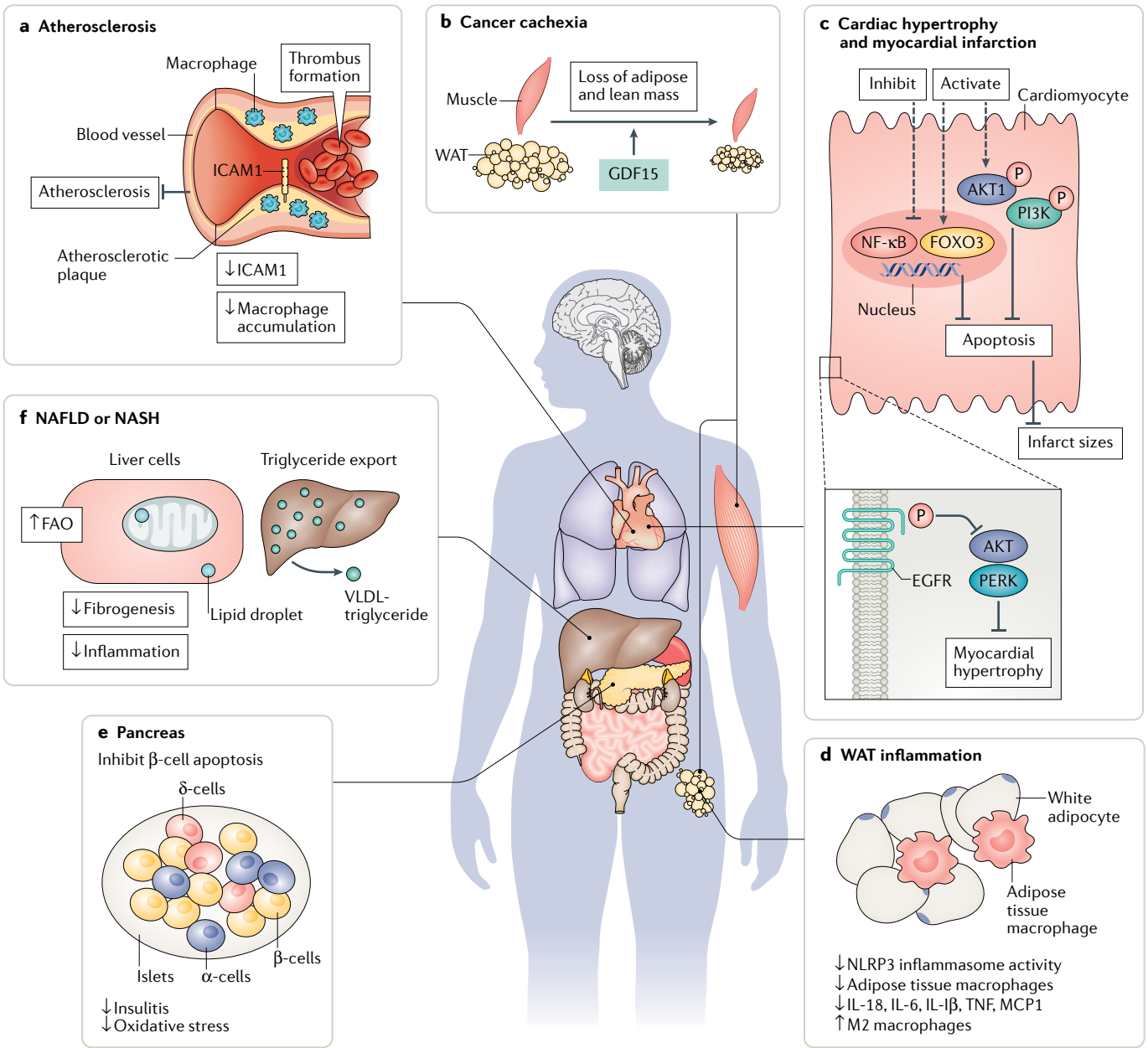

Fig. 4 | **Potential effects of GDF15 in cardiometabolic diseases beyond obesity. a** | In atherosclerotic cardiovascular disease, growth differentiation factor 15 (GDF15) might inhibit atherosclerosis by decreasing macrophage accumulation in atherosclerotic plaques and by inhibiting intercellular adhesion molecule 1 (ICAM1) expression and thrombus formation. **b** | During cancer cachexia, GDF15 secreted by tumours suppresses appetite and promotes adipose tissue lipolysis and muscle proteolysis effects, which can be blocked using neutralizing antibodies to GDF15 or glial cell-derived neurotrophic factor family receptor alpha-like (GFRAL). **c** | GDF15 might prevent the damaging effects of myocardial infarction by inhibiting apoptosis induced by ischaemia–reperfusion injury, potentially through PI3K, AKT, FOXO3 and NF-κB signalling pathways. GDF15 might also inhibit myocardial hypertrophy by inhibiting activating phosphorylation of epidermal growth factor receptor (EGFR) and downstream kinases (RAC-alpha serine–threonine-protein kinase (AKT) and extracellular signal-related kinases 1/2 (ERK1/2)). **d** | Inhibition of inflammation by GDF15 independently of reductions in body mass and/or adiposity might exert beneficial effects under multiple disease conditions, including diabetes mellitus, cardiovascular disease and non-alcoholic fatty liver disease (NAFLD). **e** | GDF15 might decrease the incidence of diabetes mellitus potentially by inhibiting insulitis, oxidative stress and β-cell apoptosis in pancreatic islets. **f** | GDF15 increases the expression of genes regulating β-oxidation and increases triglyceride export from the liver through VLDL-triglyceride, effects that might reduce inflammation and fibrosis in NAFLD independently of reductions in adiposity. The mechanisms by which GDF15 exerts effects on all these distinct cell types and disease conditions remains unclear, given that GFRAL is not abundantly expressed and will require further study. FAO, fatty acid oxidation; NASH, non-alcoholic steatohepatitis; WAT, white adipose tissue.

research investigating the role of GDF15 and GFRAL is required to understand whether increases in GDF15 promote or inhibit the development of atherosclerosis in other animal models that are potentially more relevant to human disease development (for example, hamsters or pigs).

Infarction, ischaemia–reperfusion injury and thrombus. Myocardial infarction and cerebral infarction are the sudden stoppage of blood flow to areas of the heart or brain, respectively[142,143]. This lack of blood flow can be fatal to the individual or result in considerable cell death and tissue damage due to lack of oxygen[142,143]. Furthermore, reperfusion of the area with oxygen-rich blood causes excessive damage due to the recruitment of inflammatory macrophages and the deposition of collagen[142,143]. Notably, *Gdf15*-knockout mice have greater mortality than wild-type mice after myocardial infarction, an effect attributed to greater myocardial rupture of the left ventricular free wall and haemothorax[144]. This increased mortality observed in *Gdf15*-knockout mice was rescued by the knockout of β2-integrins in myeloid cells, suggesting that GDF15 protected mice from fatal cardiac rupture by blocking chemokine-triggered leucocyte integrin activation and preventing the recruitment of neutrophils[144]. In a mouse model of ischaemia–reperfusion injury, GDF15 deficiency increased infarct sizes and cardiomyocyte apoptosis in the infarct border zone, indicating that GDF15 might protect the heart from myocardial infarcations[130]. Recombinant GDF15 also protected cultured cardiomyocytes from apoptosis during simulated ischaemia–reperfusion injury[130]. Similarly, the overexpression of GDF15 in mice prevented cold-induced ischaemia–reperfusion injury in transplanted hearts by inhibiting cell apoptosis and neutrophil infiltration[145]. Additional in vitro and mouse studies have examined the effects of GDF15 on clot formation and found that GDF15 inhibited platelet integrin activation and further prevented thrombus formation[146].

Collectively, these studies suggest that GDF15 serves a protective role in CVD as a result of infarction, ischaemia–reperfusion injury or thrombotic events; however, the cellular–protein–receptor interactions of these effects are still unclear.

Cardiac hypertrophy. Cardiac hypertrophy can precipitate the development of heart failure and is the pathological thickening or enlargement of the walls of the heart due to increased cardiomyocyte size and extracellular matrix deposition[147]. Serum levels of GDF15 in patients with hypertension are positively correlated with the thickness of the posterior wall of the left ventricle and left ventricular mass[148]. However, recombinant GDF15 treatment or the cardiac-specific overexpression of GDF15 decreases hypertrophy in cultured cardiomyocytes and attenuates cardiac ventricular dilation and heart failure in mice[129]. Although this finding was reported to involve the activation of SMAD signalling in mice[129], the activation of this pathway was not detectable in human primary cardiomyocytes treated with GDF15 (REF.[62]). In a model of norepinephrine-induced myocardial hypertrophy, GDF15 also inhibited hypertrophy through the inhibition of AKT and ERK1/2 (REF.[148]). Future studies in mice deficient for GDF15 and GFRAL are needed to determine whether GDF15 plays an important physiological or pathological role in the development of cardiac hypertrophy and to identify the potential mechanisms of action.

GDF15–GFRAL and cachexia

Cancer cachexia is a debilitating multifactorial syndrome characterized by an involuntary loss of adipose and lean mass associated with poor quality of life and reduced survival[15,149]. GDF15 is well documented to be elevated in many cancers; however, accumulating evidence indicates that it plays a critical role in driving cancer cachexia[69,150,151]. For example, the treatment of mice with monoclonal antibodies to either GDF15 (REF.[150]) or GFRAL[86] prevents body weight loss in several distinct carcinoma models. Platinum-based chemotherapies, such as cisplatin, also increase GDF15, which exacerbates cancer cachexia[150,151], an effect that can be attenuated when tumour-bearing mice are treated with a monoclonal antibody to GDF15 (REF.[22]). Importantly, a GDF15-neutralizing antibody also attenuates cisplatin-induced anorexia in non-human primates[22]. Although most of the beneficial effects of GDF15 neutralization on preventing cachexia have been ascribed to restoring food intake, a 2018 study has indicated this approach can also prevent cachexia independently of food intake by directly inhibiting WAT lipolysis[86]. Collectively, these data strongly support the clinical application of GDF15–GFRAL-neutralizing antibodies for the treatment of cancer cachexia.

Conclusions

GDF15 is a stress-responsive cytokine whose expression is elevated in obesity, T2DM, NAFLD, NASH, CVD and cancer. The primary cell types contributing to increases in GDF15 in these different diseases remains unclear; however, several studies have indicated that mitochondrial stress and activation of the integrated stress response is important. Notably, in contrast to pathological conditions, GDF15 expression is also elevated with metformin treatment and after endurance exercise, both of which exert positive health benefits. These observations suggest that associations with disease development are not causative but are potentially compensatory. Consistent with this idea, in animal models, increases in GDF15 reduce food intake and blood levels of glucose through signalling within the brainstem via the GDF15–GFRAL–RET axis. Although calorically restricted pair-feeding experiments indicate that GDF15 reduces blood levels of glucose primarily via reduced food intake[63], potential effects are exerted on islet function and on suppressing inflammation, suggesting that additional mechanisms are involved.

Indeed, many important questions remain to be answered. For example, why would GDF15 have evolved to preferentially suppress the intake of high-fat compared with low-fat foods? Other studies are needed to examine whether GDF15 exerts weight-neutral effects on blood glucose control, insulin sensitivity, and liver lipids

and/or fibrosis. Similarly, the role of GDF15 as a diagnostic biomarker for CVD is well established; however, whether it exerts therapeutic effects or increases disease severity requires further study. How GDF15–GFRAL–RET signalling regulates energy homeostasis beyond food intake also remains to be determined as does whether other physiological actions of GDF15 signalling exist beyond the regulation of food intake. Specifically, further studies on the effect of the intracellular forms of GDF15 on biological processes, such as inflammation, are needed. Little information is also available on the biological activity of non-mature GDF15 (pro-GDF15 and the pro-peptide GDF15 fragment) and a potential soluble GFRAL receptor. Understanding these relationships will be central to establishing the safety of GDF15-targeted therapies and the therapeutic potential of GDF15 in the treatment of cardiometabolic disease.

With respect to clinical translation, encouraging associations are observed between GDF15 and GFRAL with reductions in body mass and improved cardiometabolic traits; however, whether these associations will translate into this pathway being a safe drug target remains to be established. Although the observation that chronic treatment with metformin increases serum levels of GDF15 provides some assurances, only with randomized clinical trials will this information become known. To date, several pharma companies, such as Amgen, Eli Lilly, Novo Nordisk, NGM Bio and Janssen, have or are conducting clinical testing of analogues of GDF15. Based on the patent literature, these are likely to involve molecular formats where the circulatory half-life has been protracted using a fragment crystallizable-domain or human serum albumin as fusion partners or site-specific lipidation to enhance human serum albumin binding. In the coming years, it will be exciting to learn about new biological roles of GDF15 and whether the promising observations in animal models translate into a new therapy for obesity and metabolic diseases.

Published online 11 August 2021

1. Lyall, D. M. et al. Association of body mass index with cardiometabolic disease in the UK Biobank: a Mendelian randomization study. *JAMA Cardiol.* **2**, 882–889 (2017).
2. Fabbrini, E., Sullivan, S. & Klein, S. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. *Hepatology* **51**, 679–689 (2010).
3. Liu, Z. et al. Causal relationships between NAFLD, T2D and obesity have implications for disease subphenotyping. *J. Hepatol.* **73**, 263–276 (2020).
4. Friedman, S. L., Neuschwander-Tetri, B. A., Rinella, M. & Sanyal, A. J. Mechanisms of NAFLD development and therapeutic strategies. *Nat. Med.* **24**, 908–922 (2018).
5. Cai, J. et al. Nonalcoholic fatty liver disease pandemic fuels the upsurge in cardiovascular diseases. *Circ. Res.* **126**, 679–704 (2020).
6. Kumanyika, S. K. et al. Population-based prevention of obesity: the need for comprehensive promotion of healthful eating, physical activity, and energy balance: a scientific statement from American Heart Association Council on Epidemiology and Prevention, Interdisciplinary Committee for Prevention (formerly the expert panel on population and prevention science). *Circulation* **118**, 428–464 (2008).
7. Gadde, K. M., Martin, C. K., Berthoud, H. R. & Heymsfield, S. B. Obesity: pathophysiology and management. *J. Am. Coll. Cardiol.* **71**, 69–84 (2018).
8. Lockhart, S. M., Saudek, V. & O'Rahilly, S. GDF15: a hormone conveying somatic distress to the brain. *Endocr. Rev.* **41**, 610–642 (2020).
9. Hsiao, E. C. et al. Characterization of growth-differentiation factor 15, a transforming growth factor beta superfamily member induced following liver injury. *Mol. Cell Biol.* **20**, 3742–3751 (2000).
10. Coll, A. P. et al. GDF15 mediates the effects of metformin on body weight and energy balance. *Nature* **578**, 444–448 (2020).
11. Paralkar, V. M. et al. Cloning and characterization of a novel member of the transforming growth factor-beta/bone morphogenetic protein family. *J. Biol. Chem.* **273**, 13760–13767 (1998).
12. Yokoyama-Kobayashi, M., Saeki, M., Sekine, S. & Kato, S. Human cDNA encoding a novel TGF-beta superfamily protein highly expressed in placenta. *J. Biochem.* **122**, 622–626 (1997).
13. Tsai, V. W. et al. Treatment with the TGF-b superfamily cytokine MIC-1/GDF15 reduces the adiposity and corrects the metabolic dysfunction of mice with diet-induced obesity. *Int. J. Obes.* **42**, 561–571 (2018).
14. Tsai, V. W. et al. Anorexia/cachexia of chronic diseases: a role for the TGF-beta family cytokine MIC-1/GDF15. *J. Cachexia Sarcopenia Muscle* **3**, 239–243 (2012).
15. Tsai, V. W. W., Husaini, Y., Sainsbury, A., Brown, D. A. & Breit, S. N. The MIC-1/GDF15-GFRAL pathway in energy homeostasis: implications for obesity, cachexia, and other associated diseases. *Cell Metab.* **28**, 353–368 (2018).
16. Dostálová, I. et al. Increased serum concentrations of macrophage inhibitory cytokine-1 in patients with obesity and type 2 diabetes mellitus: the influence of very low calorie diet. *Eur. J. Endocrinol.* **161**, 397–404 (2009).
17. Vila, G. et al. The relationship between insulin resistance and the cardiovascular biomarker growth differentiation factor-15 in obese patients. *Clin. Chem.* **57**, 309–316 (2011).
18. Xiong, Y. et al. Long-acting MIC-1/GDF15 molecules to treat obesity: Evidence from mice to monkeys. *Sci. Transl. Med.* **9**, eaan8732 (2017).
19. Gil, C. I. et al. Role of GDF15 in active lifestyle induced metabolic adaptations and acute exercise response in mice. *Sci. Rep.* **9**, 20120 (2019).
20. Tsai, V. W. et al. Serum levels of human MIC-1/GDF15 vary in a diurnal pattern, do not display a profile suggestive of a satiety factor and are related to BMI. *PLoS One* **10**, e0133362 (2015).
21. Gerstein, H. C. et al. Growth differentiation factor 15 as a novel biomarker for metformin. *Diabetes Care* **40**, 280–283 (2017).
22. Altena, R. et al. Growth differentiation factor 15 (GDF-15) plasma levels increase during bleomycin- and cisplatin-based treatment of testicular cancer patients and relate to endothelial damage. *PLoS One* **10**, e0115372 (2015).
23. Petry, C. J. et al. Associations of vomiting and antiemetic use in pregnancy with levels of circulating GDF15 early in the second trimester: A nested case-control study. *Wellcome Open Res.* **3**, 123–123 (2018).
24. Tsai, V. W.-W. et al. TGF-b superfamily cytokine MIC-1/GDF15 is a physiological appetite and body weight regulator. *PLoS One* **8**, e55174 (2013).
25. Patel, S. et al. GDF15 provides an endocrine signal of nutritional stress in mice and humans. *Cell Metab.* **29**, 707–718.e8 (2019).
26. Campderros, L. et al. Brown adipocytes secrete GDF15 in response to thermogenic activation. *Obesity* **27**, 1606–1616 (2019).
27. Bootcov, M. R. et al. MIC-1, a novel macrophage inhibitory cytokine, is a divergent member of the TGF-beta superfamily. *Proc. Natl Acad. Sci. USA* **94**, 11514–11519 (1997).
28. Bottner, M., Suter-Crazzolara, C., Schober, A. & Unsicker, K. Expression of a novel member of the TGF-beta superfamily, growth/differentiation factor-15/macrophage-inhibiting cytokine-1 (GDF-15/MIC-1) in adult rat tissues. *Cell Tissue Res.* **297**, 103–110 (1999).
29. Liu, R. & Nikolajczyk, B. S. Tissue immune cells fuel obesity-associated inflammation in adipose tissue and beyond. *Front. Immunol.* **10**, 1587 (2019).
30. Ramachandran, P. et al. Resolving the fibrotic niche of human liver cirrhosis at single-cell level. *Nature* **575**, 512–518 (2019).
31. Govaere, O. et al. Transcriptomic profiling across the nonalcoholic fatty liver disease spectrum reveals gene signatures for steatohepatitis and fibrosis. *Sci. Transl. Med.* **12**, eaba4448 (2020).
32. Nohara, S. et al. GDF-15, a mitochondrial disease biomarker, is associated with the severity of multiple sclerosis. *J. Neurol. Sci.* **405**, 116429 (2019).
33. Wollert, K. C., Kempf, T. & Wallentin, L. Growth differentiation factor 15 as a biomarker in cardiovascular disease. *Clin. Chem.* **63**, 140–151 (2017).
34. Lindholm, D. et al. Association of multiple biomarkers with risk of all-cause and cause-specific mortality after acute coronary syndromes: a secondary analysis of the PLATO biomarker study. *JAMA Cardiol.* **3**, 1160–1166 (2018).
35. Wallentin, L. et al. GDF-15 for prognostication of cardiovascular and cancer morbidity and mortality in men. *PLoS One* **8**, e78797 (2013).
36. Brown, D. A. et al. Macrophage inhibitory cytokine 1: a new prognostic marker in prostate cancer. *Clin. Cancer Res.* **15**, 6658–6664 (2009).
37. Tsui, K. H. et al. Growth differentiation factor-15: a p53- and demethylation-upregulating gene represses cell proliferation, invasion, and tumorigenesis in bladder carcinoma cells. *Sci. Rep.* **5**, 12870 (2015).
38. Tan, M., Wang, Y., Guan, K. & Sun, Y. PTGF-β, a type β transforming growth factor (TGF-β) superfamily member, is a p53 target gene that inhibits tumor cell growth via TGF-β signaling pathway. *Proc. Natl Acad. Sci. USA* **97**, 109–114 (2000).
39. Baek, S. J., Kim, J. S., Nixon, J. B., DiAugustine, R. P. & Eling, T. E. Expression of NAG-1, a transforming growth factor-beta superfamily member, by troglitazone requires the early growth response gene EGR-1. *J. Biol. Chem.* **279**, 6883–6892 (2004).
40. Li, D., Zhang, H. & Zhong, Y. Hepatic GDF15 is regulated by CHOP of the unfolded protein response and alleviates NAFLD progression in obese mice. *Biochem. Biophys. Res. Commun.* **498**, 388–394 (2018).
41. Day, E. A. et al. Metformin-induced increases in GDF15 are important for suppressing appetite and promoting weight loss. *Nat. Metab.* **1**, 1202–1208 (2019).
42. Quiros, P. M. et al. Multi-omics analysis identifies ATF4 as a key regulator of the mitochondrial stress response in mammals. *J. Cell Biol.* **216**, 2027–2045 (2017).
43. Anderson, N. S. & Haynes, C. M. Folding the mitochondrial UPR into the integrated stress response. *Trends Cell Biol.* **30**, 428–439 (2020).
44. Chung, H. K. et al. Growth differentiation factor 15 is a myomitokine governing systemic energy homeostasis. *J. Cell Biol.* **216**, 149–165 (2017).
45. Choi, M. J. et al. An adipocyte-specific defect in oxidative phosphorylation increases systemic energy expenditure and protects against diet-induced obesity in mouse models. *Diabetologia* **63**, 837–852 (2020).
46. Zechner, C. et al. Total skeletal muscle PGC-1 deficiency uncouples mitochondrial derangements from fiber type determination and insulin sensitivity. *Cell Metab.* **12**, 633–642 (2010).

47. O'Neill, H. M. et al. AMP-activated protein kinase (AMPK) β1β2 muscle null mice reveal an essential role for AMPK in maintaining mitochondrial content and glucose uptake during exercise. *Proc. Natl Acad. Sci. USA* **108**, 16092–16097 (2011).
48. Wredenberg, A. et al. Respiratory chain dysfunction in skeletal muscle does not cause insulin resistance. *Biochem. Biophys. Res. Commun.* **350**, 202–207 (2006).
49. Pospisilik, J. A. et al. Targeted deletion of AIF decreases mitochondrial oxidative phosphorylation and protects from obesity and diabetes. *Cell* **131**, 476–491 (2007).
50. Conte, M. et al. Human aging and longevity are characterized by high levels of mitokines. *J. Gerontol. A Biol. Sci. Med. Sci.* **74**, 600–607 (2019).
51. Moon, J. S. et al. Growth differentiation factor 15 protects against the aging-mediated systemic inflammatory response in humans and mice. *Aging Cell* **19**, e13195 (2020).
52. Fujita, Y., Taniguchi, Y., Shinkai, S., Tanaka, M. & Ito, M. Secreted growth differentiation factor 15 as a potential biomarker for mitochondrial dysfunctions in aging and age-related disorders. *Geriatr. Gerontol. Int.* **16**, 17–29 (2016).
53. Townsend, L. K. et al. AMPK mediates energetic stress-induced liver GDF15. *FASEB J.* **35**, e21218 (2021).
54. Baek, S. J. & Eling, T. Growth differentiation factor 15 (GDF15): a survival protein with therapeutic potential in metabolic diseases. *Pharmacol. Ther.* **198**, 46–58 (2019).
55. Li, J. J. et al. Growth differentiation factor 15 maturation requires proteolytic cleavage by PCSK3, -5, and -6. *Mol. Cell Biol.* **38**, e00249-18 (2018).
56. Zhang, Z. et al. ARRB1 inhibits non-alcoholic steatohepatitis progression by promoting GDF15 maturation. *J. Hepatol.* **72**, 976–989 (2020).
57. Bauskin, A. R. et al. The propeptide mediates formation of stromal stores of PROMIC-1: role in determining prostate cancer outcome. *Cancer Res.* **65**, 2330–2336 (2005).
58. Bauskin, A. R. et al. The propeptide of macrophage inhibitory cytokine (MIC-1), a TGF-beta superfamily member, acts as a quality control determinant for correctly folded MIC-1. *EMBO J.* **19**, 2212–2220 (2000).
59. Li, S. et al. Maturation of growth differentiation factor 15 in human placental trophoblast cells depends on the interaction with matrix metalloproteinase-26. *J. Clin. Endocrinol. Metab.* **99**, E2277–E2287 (2014).
60. Abd El-Aziz, S. H., Endo, Y., Miyamaori, H., Takino, T. & Sato, H. Cleavage of growth differentiation factor 15 (GDF15) by membrane type 1-matrix metalloproteinase abrogates GDF15-mediated suppression of tumor cell growth. *Cancer Sci.* **98**, 1330–1335 (2007).
61. Hsu, J. Y. et al. Non-homeostatic body weight regulation through a brainstem-restricted receptor for GDF15. *Nature* **550**, 255–259 (2017).
62. Yang, L. et al. GFRAL is the receptor for GDF15 and is required for the anti-obesity effects of the ligand. *Nat. Med.* **23**, 1158–1166 (2017).
63. Mullican, S. E. et al. GFRAL is the receptor for GDF15 and the ligand promotes weight loss in mice and nonhuman primates. *Nat. Med.* **23**, 1150–1157 (2017).
64. Emmerson, P. J. et al. The metabolic effects of GDF15 are mediated by the orphan receptor GFRAL. *Nat. Med.* **23**, 1215–1219 (2017).
65. Wong, A. et al. Phosphotyrosine 1062 is critical for the in vivo activity of the Ret9 receptor tyrosine kinase isoform. *Mol. Cell. Biol.* **25**, 9661–9673 (2005).
66. Tsai, V. W. et al. The anorectic actions of the TGFbeta cytokine MIC-1/GDF15 require an intact brainstem area postrema and nucleus of the solitary tract. *PLoS One* **9**, e100370 (2014).
67. Codo, P. et al. Control of glioma cell migration and invasiveness by GDF-15. *Oncotarget* **7**, 7732–7746 (2016).
68. Olsen, O. E., Skjaervik, A., Stordal, B. F., Sundan, A. & Holien, T. TGF-beta contamination of purified recombinant GDF15. *PLoS One* **12**, e0187349 (2017).
69. Johnen, H. et al. Tumor-induced anorexia and weight loss are mediated by the TGF-β superfamily cytokine MIC-1. *Nat. Med.* **13**, 1333–1340 (2007).
70. Tsai, V. W.-W. et al. GDF15 mediates adiposity resistance through actions on GFRAL neurons in the hindbrain AP/NTS. *Int. J. Obes.* **43**, 2370–2380 (2019).
71. Frikke-Schmidt, H. et al. GDF15 acts synergistically with liraglutide but is not necessary for the weight loss induced by bariatric surgery in mice. *Mol. Metab.* **21**, 13–21 (2019).
72. Murphy, K. G. & Bloom, S. R. Gut hormones and the regulation of energy homeostasis. *Nature* **444**, 854–859 (2006).
73. Banno, R. et al. Central administration of melanocortin agonist increased insulin sensitivity in diet-induced obese rats. *FEBS Lett.* **581**, 1131–1136 (2007).
74. Zarjevski, N., Cusin, I., Vettor, R., Rohner-Jeanrenaud, F. & Jeanrenaud, B. Intracerebroventricular administration of neuropeptide Y to normal rats has divergent effects on glucose utilization by adipose tissue and skeletal muscle. *Diabetes* **43**, 764–769 (1994).
75. Worth, A. A. et al. The cytokine GDF15 signals through a population of brainstem cholecystokinin neurons to mediate anorectic signalling. *eLife* **9**, e55164 (2020).
76. Camilleri, M. Peripheral mechanisms in the control of appetite and related experimental therapies in obesity. *Regul. Pept.* **156**, 24–27 (2009).
77. Borner, T. et al. GDF15 induces anorexia through nausea and emesis. *Cell Metab.* **31**, 351–362.e355 (2020).
78. Endo, T. et al. Neurochemistry and neuropharmacology of emesis - the role of serotonin. *Toxicology* **153**, 189–201 (2000).
79. Herrstedt, J. The latest consensus on antiemetics. *Curr. Opin. Oncol.* **30**, 233–239 (2018).
80. Hayes, M. R. & Covasa, M. Gastric distension enhances CCK-induced Fos-like immunoreactivity in the dorsal hindbrain by activating 5-HT3 receptors. *Brain Res.* **1088**, 120–130 (2006).
81. Borner, T. et al. GDF15 induces an aversive visceral malaise state that drives anorexia and weight loss. *Cell Rep.* **31**, 107543 (2020).
82. Palmiter, R. D. The parabrachial nucleus: CGRP neurons function as a general alarm. *Trends Neurosci.* **41**, 280–293 (2018).
83. Morton, G. J., Meek, T. H. & Schwartz, M. W. Neurobiology of food intake in health and disease. *Nat. Rev. Neurosci.* **15**, 367–378 (2014).
84. Carter, M. E., Soden, M. E., Zweifel, L. S. & Palmiter, R. D. Genetic identification of a neural circuit that suppresses appetite. *Nature* **503**, 111–114 (2013).
85. Chrysovergis, K. et al. NAG-1/GDF-15 prevents obesity by increasing thermogenesis, lipolysis and oxidative metabolism. *Int. J. Obes.* **38**, 1555–1564 (2014).
86. Suriben, R. et al. Antibody-mediated inhibition of GDF15–GFRAL activity reverses cancer cachexia in mice. *Nat. Med.* **26**, 1264–1270 (2020).
87. Beiroa, D. et al. GLP-1 agonism stimulates brown adipose tissue thermogenesis and browning through hypothalamic AMPK. *Diabetes* **63**, 3346–3358 (2014).
88. Tomas, E. et al. GLP-1(32-36)amide pentapeptide increases basal energy expenditure and inhibits weight gain in obese mice. *Diabetes* **64**, 2409–2419 (2015).
89. Heppner, K. M. et al. Contribution of brown adipose tissue activity to the control of energy balance by GLP-1 receptor signalling in mice. *Diabetologia* **58**, 2124–2132 (2015).
90. Harder, H., Nielsen, L., Tu, D. T. & Astrup, A. The effect of liraglutide, a long-acting glucagon-like peptide 1 derivative, on glycemic control, body composition, and 24-h energy expenditure in patients with type 2 diabetes. *Diabetes Care* **27**, 1915–1921 (2004).
91. Müller, T. D. et al. Glucagon-like peptide 1 (GLP-1). *Mol. Metab.* **30**, 72–130 (2019).
92. Yengo, L. et al. Meta-analysis of genome-wide association studies for height and body mass index in approximately 700000 individuals of European ancestry. *Hum. Mol. Genet.* **27**, 3641–3649 (2018).
93. Pulit, S. L. et al. Meta-analysis of genome-wide association studies for body fat distribution in 694 649 individuals of European ancestry. *Hum. Mol. Genet.* **28**, 166–174 (2019).
94. Võsa, U. et al. Unraveling the polygenic architecture of complex traits using blood eQTL metaanalysis. *bioRxiv* https://doi.org/10.1101/447367 (2018).
95. Sun, B. B. et al. Genomic atlas of the human plasma proteome. *Nature* **558**, 73–79 (2018).
96. Lonsdale, J. et al. The genotype-tissue expression (GTEx) project. *Nat. Genet.* **45**, 580–585 (2013).
97. Machiela, M. J. & Chanock, S. J. LDlink: a web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. *Bioinformatics* **31**, 3555–3557 (2015).
98. Turcot, V. et al. Protein-altering variants associated with body mass index implicate pathways that control energy intake and expenditure in obesity. *Nat. Genet.* **50**, 26–41 (2018).
99. Li, J. et al. Cryo-EM analyses reveal the common mechanism and diversification in the activation of RET by different ligands. *eLife* **8**, e47650 (2019).
100. Cheung, C. L., Tan, K. C. B., Au, P. C. M., Li, G. H. Y. & Cheung, B. M. Y. Evaluation of GDF15 as a therapeutic target of cardiometabolic diseases in human: A Mendelian randomization study. *EBioMedicine* **41**, 85–90 (2019).
101. Macia, L. et al. Macrophage inhibitory cytokine 1 (MIC-1/GDF15) decreases food intake, body weight and improves glucose tolerance in mice on normal & obesogenic diets. *PLoS One* **7**, e34868 (2012).
102. Wang, X. et al. hNAG-1 increases lifespan by regulating energy metabolism and insulin/IGF-1/mTOR signaling. *Aging* **6**, 690–704 (2014).
103. Wang, X., Chrysovergis, K., Kosak, J. & Eling, T. E. Lower NLRP3 inflammasome activity in NAG-1 transgenic mice is linked to a resistance to obesity and increased insulin sensitivity. *Obesity* **22**, 1256–1263 (2014).
104. Hong, J. H. et al. GDF15 is a novel biomarker for impaired fasting glucose. *Diabetes Metab. J.* **38**, 472–479 (2014).
105. Kempf, T. et al. Growth differentiation factor 15 predicts future insulin resistance and impaired glucose control in obese nondiabetic individuals: results from the XENDOS trial. *Eur. J. Endocrinol.* **167**, 671–678 (2012).
106. Carstensen, M. et al. Macrophage inhibitory cytokine-1 is increased in individuals before type 2 diabetes diagnosis but is not an independent predictor of type 2 diabetes: the Whitehall II study. *Eur. J. Endocrinol.* **162**, 913–917 (2010).
107. Schernthaner-Reiter, M. H. et al. GDF15 reflects beta cell function in obese patients independently of the grade of impairment of glucose metabolism. *Nutr. Metab. Cardiovasc. Dis.* **29**, 334–342 (2019).
108. Nakayasu, E. S. et al. Comprehensive proteomics analysis of stressed human islets identifies GDF15 as a target for type 1 diabetes intervention. *Cell Metab.* **31**, 363–374.e6 (2020).
109. Yi, H. S. et al. T-cell senescence contributes to abnormal glucose homeostasis in humans and mice. *Cell Death Dis.* **10**, 249 (2019).
110. Jung, S. B. et al. Reduced oxidative capacity in macrophages results in systemic insulin resistance. *Nat. Commun.* **9**, 1551 (2018).
111. Lee, S. E. et al. Growth differentiation factor 15 mediates systemic glucose regulatory action of T-helper type 2 cytokines. *Diabetes* **66**, 2774–2788 (2017).
112. Foretz, M., Guigas, B. & Viollet, B. Understanding the glucoregulatory mechanisms of metformin in type 2 diabetes mellitus. *Nat. Rev. Endocrinol.* **15**, 569–589 (2019).
113. Steinberg, G. R. Cellular energy sensing and metabolism—implications for treating diabetes: the 2017 outstanding scientific achievement award lecture. *Diabetes* **67**, 169–179 (2018).
114. Natali, A. et al. Metformin is the key factor in elevated plasma growth differentiation factor-15 levels in type 2 diabetes: a nested, case-control study. *Diabetes Obes. Metab.* **21**, 412–416 (2019).
115. Colberg, S. R. et al. Physical activity/exercise and diabetes: a position statement of the American Diabetes Association. *Diabetes Care* **39**, 2065–2079 (2016).
116. Kleinert, M. et al. Exercise increases circulating GDF15 in humans. *Mol. Metab.* **9**, 187–191 (2018).
117. Tchou, I. et al. Growth-differentiation factor-15, endoglin and N-terminal pro-brain natriuretic peptide induction in athletes participating in an ultramarathon foot race. *Biomarkers* **14**, 418–422 (2009).
118. Zhang, H., Fealy, C. E. & Kirwan, J. P. Exercise training promotes a GDF15-associated reduction in fat mass in older adults with obesity. *Am. J. Physiol. Endocrinol. Metab.* **316**, E829–E836 (2019).
119. Laurens, C. et al. Growth and differentiation factor 15 is secreted by skeletal muscle during exercise and promotes lipolysis in humans. *JCI insight* **5**, e131870 (2020).
120. Klein, A. B. et al. Pharmacological but not physiological GDF15 suppresses feeding and the motivation to exercise. *Nat. Commun.* **12**, 1041 (2021).
121. Yardley, M. et al. Immediate response in markers of inflammation and angiogenesis during exercise: a randomised cross-over study in heart transplant recipients. *Open Heart* **4**, e000635 (2017).

122. Murphy, R. M., Watt, M. J. & Febbraio, M. A. Metabolic communication during exercise. *Nat. Metab.* **2**, 805–816 (2020).
123. Camacho, R. C., Donahue, E. P., James, F. D., Berglund, E. D. & Wasserman, D. H. Energy state of the liver during short-term and exhaustive exercise in C57BL/6J mice. *Am. J. Physiol. Endocrinol. Metab.* **290**, E405–E408 (2006).
124. Kim, K. H. et al. Growth differentiation factor 15 ameliorates nonalcoholic steatohepatitis and related metabolic disorders in mice. *Sci. Rep.* **8**, 6789 (2018).
125. Koo, B. K. et al. Growth differentiation factor 15 predicts advanced fibrosis in biopsy-proven non-alcoholic fatty liver disease. *Liver Int.* **38**, 695–705 (2018).
126. Chung, H. K. et al. GDF15 deficiency exacerbates chronic alcohol- and carbon tetrachloride-induced liver injury. *Sci. Rep.* **7**, 17238 (2017).
127. Luan, H. H. et al. GDF15 is an inflammation-induced central mediator of tissue tolerance. *Cell* **178**, 1231–1244.e11 (2019).
128. Adela, R. & Banerjee, S. K. GDF-15 as a target and biomarker for diabetes and cardiovascular diseases: a translational prospective. *J. Diabetes Res.* **2015**, 490842 (2015).
129. Xu, J. et al. GDF15/MIC-1 functions as a protective and antihypertrophic factor released from the myocardium in association with SMAD protein activation. *Circ. Res.* **98**, 342–350 (2006).
130. Kempf, T. et al. The transforming growth factor-beta superfamily member growth-differentiation factor-15 protects the heart from ischemia/reperfusion injury. *Circ. Res.* **98**, 351–360 (2006).
131. Kempf, T. & Wollert, K. C. Growth differentiation factor-15: a new biomarker in cardiovascular disease. *Herz* **34**, 594–599 (2009).
132. Lusis, A. J. Atherosclerosis. *Nature* **407**, 233–241 (2000).
133. Wang, D. et al. Targeting foam cell formation in atherosclerosis: therapeutic potential of natural products. *Pharmacol. Rev.* **71**, 596–670 (2019).
134. Bentzon, J. F., Otsuka, F., Virmani, R. & Falk, E. Mechanisms of plaque formation and rupture. *Circ. Res.* **114**, 1852–1866 (2014).
135. Bonaterra, G. A. et al. Growth differentiation factor-15 deficiency inhibits atherosclerosis progression by regulating interleukin-6-dependent inflammatory response to vascular injury. *J. Am. Heart Assoc.* **1**, e002550 (2012).
136. de Jager, S. C. et al. Growth differentiation factor 15 deficiency protects against atherosclerosis by attenuating CCR2-mediated macrophage chemotaxis. *J. Exp. Med.* **208**, 217–225 (2011).
137. Preusch, M. R. et al. GDF-15 protects from macrophage accumulation in a mousemodel of advanced atherosclerosis. *Eur. J. Med. Res.* **18**, 19 (2013).
138. Johnen, H. et al. Increased expression of the TGF-b superfamily cytokine MIC-1/GDF15 protects ApoE(-/-) mice from the development of atherosclerosis. *Cardiovasc. Pathol.* **21**, 499–505 (2012).
139. Tian, X. Y. et al. Thermoneutral housing accelerates metabolic inflammation to potentiate atherosclerosis but not insulin resistance. *Cell Metab.* **23**, 165–178 (2016).
140. Skop, V. et al. Mouse thermoregulation: introducing the concept of the thermoneutral point. *Cell Rep.* **31**, 107501 (2020).
141. Silvestre-Roig, C., Braster, Q., Ortega-Gomez, A. & Soehnlein, O. Neutrophils as regulators of cardiovascular inflammation. *Nat. Rev. Cardiol.* **17**, 327–340 (2020).
142. Thygesen, K. et al. Third universal definition of myocardial infarction. *Nat. Rev. Cardiol.* **9**, 620–633 (2012).
143. Saver, J. L. Proposal for a universal definition of cerebral infarction. *Stroke* **39**, 3110–3115 (2008).
144. Kempf, T. et al. GDF-15 is an inhibitor of leukocyte integrin activation required for survival after myocardial infarction in mice. *Nat. Med.* **17**, 581–588 (2011).
145. Zhang, Y. et al. Over-expression of growth differentiation factor 15 (GDF15) preventing cold ischemia reperfusion (I/R) injury in heart transplantation through Foxo3a signaling. *Oncotarget* **8**, 36531–36544 (2017).
146. Rossaint, J., Vestweber, D. & Zarbock, A. GDF-15 prevents platelet integrin activation and thrombus formation. *J. Thromb. Haemost.* **11**, 335–344 (2013).
147. Sen-Chowdhry, S., Jacoby, D., Moon, J. C. & McKenna, W. J. Update on hypertrophic cardiomyopathy and a guide to the guidelines. *Nat. Rev. Cardiol.* **13**, 651–675 (2016).
148. Xu, X. Y. et al. Growth differentiation factor (GDF)-15 blocks norepinephrine-induced myocardial hypertrophy via a novel pathway involving inhibition of epidermal growth factor receptor transactivation. *J. Biol. Chem.* **289**, 10084–10094 (2014).
149. Tisdale, M. J. Molecular pathways leading to cancer cachexia. *Physiology* **20**, 340–348 (2005).
150. Lerner, L. et al. MAP3K11/GDF15 axis is a critical driver of cancer cachexia. *J. Cachexia, Sarcopenia Muscle* **7**, 467–482 (2016).
151. Breen, D. M. et al. GDF-15 neutralization alleviates platinum-based chemotherapy-induced emesis, anorexia, and weight loss in mice and nonhuman primates. *Cell Metab.* **32**, 938–950.e6 (2020).
152. Tran, T., Yang, J., Gardner, J. & Xiong, Y. GDF15 deficiency promotes high fat diet-induced obesity in mice. *PLoS One* **13**, e0201584 (2018).

Acknowledgements
G.R.S. acknowledges the support of a Diabetes Canada Investigator Award (DI-5-17-5302-GS), a Canadian Institutes of Health Research Foundation Grant (201709FDN-CEBA-116200), a Tier 1 Canada Research Chair in Metabolic Diseases and a J. Bruce Duncan Endowed Chair in Metabolic Diseases. L.K.T. acknowledges the support of a CIHR Post-Doctoral Fellowship Award and Michael DeGroote Fellowship Award in Basic Biomedical Science.

Author contributions
D.W. researched data for the article. All authors made substantial contributions to the discussion of content, wrote the article, and reviewed and/or edited the manuscript before submission.

Competing interests
D.D. and S.B.J. are employees of Novo Nordisk A/S, a pharmaceutical company producing and selling medicine for the treatment of diabetes and obesity. G.R.S. is a co-founder and shareholder of Espervita Therapeutics, a company developing new medications for liver cancer. McMaster University has received funding from Espervita Therapeutics, Esperion Therapeutics, Poxel Pharmaceuticals and Novo Nordisk for research conducted in the laboratory of G.R.S. G.R.S. has received consulting/speaking fees from Astra Zeneca, Eli Lilly, Esperion Therapeutics, Merck, Poxel Pharmaceuticals and Takeda. The other authors declare no competing interests.

Peer review information
Nature Reviews Endocrinology thanks S. Breit, S. O'Rahilly and M. Shong for their contribution to the peer review of this work.

RELATED LINKS
Common Metabolic Diseases Knowledge Portal: https://t2d.hugeamp.org/region.html?chr=19&end=18549986&phenotype=WHRadjBMI&start=18435541
The Blood Atlas: https://www.proteinatlas.org/humanproteome/blood
the Human Protein Atlas GFRAL expression: https://www.proteinatlas.org/ENSG00000187871-GFRAL/blood