MOLECULAR
METABOLISM

Growth differentiation factor 15 as a potential therapeutic for treating obesity

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ABSTRACT

Background: Obesity is rapidly becoming one of the world's most critical health care concerns. Comorbidities accompanying excess weight include cardiovascular disease, diabetes, and certain cancers. These comorbidities result in greater hospitalization and other health care-related costs. Economic impacts are likely to be felt more acutely in developing countries, where obesity rates continue to rise and health care resources are already insufficient. Some of the more effective treatments are invasive and expensive surgeries, which some economies in the world cannot afford to offer to a broad population. Pharmacological therapies are needed to supplement treatment options for patients who cannot, or will not, undergo surgical treatment. However, the few drug therapies currently available have either limited efficacy or safety concerns. A possible exception has been glucagon-like peptide-1 analogs, although these have shown a number of adverse events. New drug therapies that are safe and produce robust weight loss are needed.

Scope of review: Herein, we review the role of growth differentiation factor 15 (GDF15) in feeding behavior and obesity, summarize some of the new and exciting biological discoveries around signaling pathways and tissue sites of action, and highlight initial efforts to develop GDF15-based therapies suitable for inducing weight loss in humans.

Major conclusions: Within the last several years, great strides have been made in understanding the biology of GDF15. Recent developments include identification of an endogenous receptor, biological localization of the receptor system, impact on energy homeostasis, and identification of molecules suitable for administration to humans as anti-obesity treatments. New and exciting research on GDF15 suggests that it holds promise as a novel obesity treatment as new molecules progress toward clinical development.

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1. INTRODUCTION

Obesity and its comorbidities have become a worldwide public health concern [1]. Although simple exercise and a properly balanced diet can be effective at maintaining a healthy body weight [2], patient adherence to these lifelong behaviors is poor. Roux-en-Y gastric bypass surgery, which involves reducing the size of the stomach and bypassing a portion of the small intestine, is one of the most radical, yet most effective, treatments for weight loss [3]. Although this procedure results in a marked weight loss, it is an invasive, complex surgery that can lead to undesirable side effects; therefore, safe and effective long-term pharmacological therapies are needed [4].

Biological pathways that can be targeted with pharmacological therapies are needed as alternatives to surgical approaches to treat obese patients. Two groups of approved drugs can be used to manage weight in patients with obesity: medications approved for obesity and medications that affect body weight for obese patients who have complications from their obesity [5]. All of these drugs have different ranges of effectiveness on weight and cause undesirable adverse effects. Therefore, clinicians and patients need pharmacological therapies that

are safe and efficacious and can be administered over the long term. One important area of investigation is the potential to target macrophage inhibitory cytokine 1/growth differentiation factor 15 (MIC-1/GDF15), referred to as GDF15 in this review, for treating obesity. GDF15 is a loosely associated member of the transforming growth factor β (TGFβ) superfamily [6—9]. Similar to other members of the TGFβ superfamily, GDF15 is a homodimer cysteine knot protein containing one interchain and eight intrachain disulfide bonds [10,11]. GDF15 is a stress-activated cytokine that during physiological conditions is found at high levels only in the placenta [12]. Elevated serum levels of GDF15 are measured in many pathological conditions such as cancer, metabolic disorders, and cardiovascular disorders and may be regarded as a common marker of disease and mortality [13—15]. GDF15 is proposed to act as an anti-inflammatory molecule. For example, GDF15 has shown anti-inflammatory properties by inhibiting leukocyte integrin activation required for survival of mice after myocardial infarction [16]. Studies with genetically engineered mice, particularly in the obesity setting, support the role of GDF15 as a regulator of body weight [17,18]. For example, depletion of GDF15 resulted in increased body weight [17], whereas overexpression

List of abbreviations		GRAL	GDNF receptor alpha-like
		HFD	high-fat diet
AAV	adeno-associated virus	HSA	human serum albumin
AP	area postrema	MIC-1	macrophage inhibitory cytokine 1
BMI	body mass index	NAG-1	non-steroidal anti-inflammatory drug activated gene
CTZ	chemoreceptor trigger zone	PDF	prostate-derived factor
DIO	diet-induced obesity	PLAB	placental bone morphogenic protein
GDF15	growth differentiation factor 15	PTGFB	platelet-derived growth factor subunit B
GDNF	glial cell-derived neurotrophic factor	RET	rearranged during transfection
GFRAL	glial cell-derived neurotrophic factor receptor alpha-like	rh	recombinant human
GLP-1	glucagon-like peptide-1	rm	recombinant murine
GPI	glycosylphosphatidylinositol	TGFβ	transforming growth factor β

resulted in mice with lower body weight and fat mass and an overall improvement in metabolic parameters [17,19,20]. Although our review focuses on the potential role of GDF15 for weight management, GDF15 has also been studied in relation to other diseases. Overall, the role of GDF15 in other diseases has been an enigma [21], and in some studies, confusion has been caused by the use of commercial recombinant material that is contaminated with other biologically active proteins, most notably TGFβ [22]. This concern is not relevant to the obesity research referenced in the rest of this review because of recombinant material being produced internally by each investigator and the purity being carefully monitored.

More recently, a clearer picture of the mechanism of action of GDF15 has emerged. Multiple research groups have described the receptor complex of GDF15 [23—26]. Although GDF15 was previously thought to signal through a canonical TGFβ pathway, it turned out to be through a glial cell line-derived neurotrophic factor (GDNF)-like alpha-1 (glial cell-derived neurotrophic factor receptor alpha-like [GFRAL])/rearranged during transfection (RET) co—receptor complex [23—26]. This complex is more related to the GDNF receptor complex GFR alpha 1—4/RET than the TGFβ pathway. In addition, solid evidence of GFRAL/RET tissue expression indicates that GFRAL/RET has a unique expression pattern, appearing only in a discrete region of the hindbrain called the area postrema (AP) and part of the nucleus of the solitary tract [23—26]. As discussed in more detail later in this review, these areas are known to receive and send signals via the vagus nerve, and ablations of these same regions render mice unresponsive to the anorectic actions of GDF15 [27]. These findings suggest that GDF15 may have an important role in mediating gut-brain communication. There is some evidence of GDF15 binding along the length of the gut, but those receptors appear to be scattered and not clustered as densely as in the hindbrain [28]; further investigation is necessary to confirm the findings regarding the receptor location in the gut. Overall, solid evidence supports the hindbrain as the sole receptor expression area.

Over the last decade, researchers in the pharmaceutical industry have tried to identify GDF15 molecules that could be used as anti-obesity treatments [23—26,28]. Several recently published reviews have broadly covered GDF15 biology and its associated disease pathologies [29,30]. Herein, we summarize GDF15 biology specifically as it relates to obesity and the potential for developing treatments for obesity. We review the basic protein characteristics, tissue expression patterns, and GDF15 receptor signaling and their relationship to GDF15's function in the brain. In addition, we summarize what both human and rodent genetics can teach us about GDF15's disease associations. We describe the current state of knowledge about GDF15's use as a potential obesity therapy and its general risks.

2. GDF15 PROCESSING, EXPRESSION, BIOCHEMISTRY, AND RECEPTOR SIGNALING

Historically, GDF15 has had several aliases: MIC-1 [6], non-steroidal anti-inflammatory drug-activated gene (NAG-1) [31], placental bone morphogenic protein (PLAB) [7], platelet-derived growth factor subunit B (PTGFB) [32], and prostate-derived factor (PDF) [9]. GDF15 has long been regarded as a distant member of the TGFβ superfamily, sharing several structurally related features [33]. It is found in the circulation as a 224 amino acid, 25 kDa, cystine knot, and homodimer protein, with <30% homology to other TGFβ family members [6]. Features shared with TGFβ family members include seven conserved cystine residues, a crystal structure that resembles other TGFβ family members by forming a homodimer that is stabilized by an interchain disulfide bond. This overall structure results in a very stable protein [24,28]. Human GDF15 protein is encoded by a 2-exon gene located on chromosome 19p13.11. Similar to most members of the TGFβ family, mature GDF15 is cleaved from a propeptide by furin-like proteases, with mature GDF15 then entering the circulation [12,34]. Circulating levels of GDF15 vary depending on factors such as age, pregnancy, weight status, and multiple disease conditions [21,32]. For example, in healthy individuals, circulating GDF15 levels can vary from 150 to 1000 pg/mL [35]. However, in conditions of inflammation, malignancy, and other stressors, serum GDF15 levels can rise to 15,000 to 20,000 pg/mL [36—38]. In humans, GDF15 is produced in a variety of tissues, most notably by the placental trophoblasts, prostate, liver, and kidney [32]. Expression from other tissues increases depending on stress or disease state [21]. The dramatic rise in GDF15 early in pregnancy while the placental area is expanding is an intriguing finding [39]. Many early trimester pregnancies are associated with morning sickness characterized by bouts of nausea and vomiting. GDF15 levels have been associated with severity of vomiting during pregnancy as well as incidence of miscarriage [40,41]. GDF15 has also been proposed as a biomarker to predict a full-term pregnancy [42].

A breakthrough came in 2017 when, in rapid succession, four groups from the pharmaceutical industry published clear and convincing data that GFRAL was indeed the long-sought receptor for GDF15 [23—26]. Coupled with its co-receptor, RET, GFRAL/RET initiates ligand-induced downstream intracellular signal cascades. Homodimeric GDF15 molecules bind to their cognate GFRAL receptors. The GDF15-GFRAL complex combines two RET molecules, forming the ternary receptor

complex GDF15-GFRAL-RET. Signaling through RET is initiated by trans-phosphorylation of specific tyrosine residues. Once the ternary complex forms, downstream effectors include Akt, ERK1/2, and PLCγ. Additionally, systemic GDF15 administration has been shown to activate c-Fos in the brain's AP, which is an indicator of recent neuronal activity [27,28]. Contrary to earlier reports, the GDF15-activated receptor complex does not result in SMAD phosphorylation [24,26]. This system closely resembles the cognate pair that GDNF and its other family members use, GFR alpha 1—4/RET, a system that initially was also elusive and was identified in 1996 [43]. RET has long been known as a proto-oncogene that codes for a receptor tyrosine kinase [44—46]. GFRAL was originally identified in 2005 as the gene called GDNF receptor alpha-like (GRAL) [47]. The GFRAL sequence is roughly 20—30% identical in sequence to the GFR alpha family of receptors, placing it as a distant member of the GDNF receptor family [47]. At the time of its cloning, an endogenous ligand for GFRAL was still unidentified. GFRAL contains a transmembrane domain with a short 23 amino acid cytoplasmic tail, as opposed to GFR alpha members that are glycosylphosphatidylinositol (GPI)-anchored proteins [47]. There is no evidence that GFRAL itself initiates downstream signaling; it must be paired with RET for that to occur [26]. GFRAL has been shown to have an extremely restricted tissue expression pattern, solely found in the hindbrain, in the AP and nucleus of the solitary tract [23—26]. No other tissue has overwhelming evidence of expression.

3. GDF15 MECHANISMS THAT UNDERLIE FEEDING BEHAVIORS AND GASTRIC EMPTYING

Prior to humans developing a centralized agrarian society, food was obtained through foraging. Depending on environmental conditions (e.g., drought or seasonal changes), these hunter-gatherer units moved from place to place in search of food. This ultimately led to encounters with new, unfamiliar plant species. Ingestion of toxic, unfamiliar plant material could have led to fatal poisonings. In addition, foods that were not toxic to a healthy adult female might contain teratogens harmful to a developing fetus [48]. A mechanism evolved to create food-induced nausea accompanied by vomiting to expel the toxic substance from the body [49]. This mechanism is thought to involve an area of the hindbrain known as the chemoreceptor trigger zone (CTZ) in the AP region [49]. The AP resides outside of the blood—brain barrier, where it is exposed to the systemic circulation and cerebrospinal fluid [50]. This allows chemoreceptors in the AP to monitor the circulation for any potentially toxic substances [51]. If certain toxin thresholds are exceeded, the AP induces nausea followed by vomiting and a longer-lasting taste aversion response [50,51] that acts as a safeguard against future ingestion of toxic material. These physiological responses are largely mediated through the vagus nerve, which directly innervates the AP [52,53]. Vagal efferent and afferent signals also play important roles in mechanisms such as postprandial gastric emptying. In fact, severing of the vagus nerve below the diaphragm leads to hypophagia and lowered body weight [54,55]. It has been demonstrated that GDF15 delays gastric emptying and vagotomy attenuates that response [28], lending support to the association between GDF15's mechanism and the CTZ and AP areas. GDF15 has also been implicated as a driver of hyperemesis gravidarum, which is observed in some pregnancies [40]. Overall, GDF15 appears to be an integral part of brain systems involved in feeding behavior but also nausea, vomiting, taste aversion, and gastric emptying.

Previous studies demonstrated a widespread hindbrain activation of c-Fos upon GDF15 administration, encompassing not only the AP but also the dorsal motor nucleus of the vagus and the nucleus of the solitary tract [27,28]. Ablation of the AP and nucleus of the solitary tract resulted in exogenously administered GDF15 losing its anorexigenic effect [27]. An earlier study showed evidence of c-Fos activation reaching into hypothalamic areas after GDF15 administration [56]. This is not surprising because the AP, and the CTZ in general, has been characterized as a crucial regulator that mediates signaling into deeper areas of the brain [57], implying that gustatory signals travel along afferent vagal fibers and input into the AP. These signals are coordinated via the AP and project into the hypothalamus, influencing neurohormone release and impacting satiety.

Other evidence further supports the AP as a food intake regulator. Lesioning of the AP in rats with Leydig cell tumors reverses declines in food intake, body weight, and food preference that normally accompany tumor growth [58]. As previously noted, GDF15 levels rise in multiple tumor settings [36,37,59]. When evidence emerged identifying GFRAL as the cognate GDF15 receptor and its location of expression was found solely in the AP, it confirmed the anorectic action of GDF15 [23,24]. Much of GDF15's actions in mice, such as delays in gastric emptying, taste aversion, reduced food intake, and cachexia, are consistent with a central action through the AP [28,56]. Activation of AP neurons by GDF15 has also been shown to activate other brain centers such as the hypothalamus, parabrachial nucleus, and amygdala, suggesting a broader, coordinated central effect of GDF15 [24,56].

The identification of amygdala activation by GDF15 [24] is a new and interesting finding. The amygdala is a complex brain structure that regulates a variety of behavioral and emotional responses [60]. As a center for emotion, the amygdala may drive reward-mediated feeding behavior as opposed to an energy-driven satiety effect [61]. It has been demonstrated in rats that amygdala lesions induce obesity and preference for a carbohydrate-rich diet [62]. Along with other structures comprising the limbic system, hippocampus, and hypothalamus, these interconnected nuclei are critically involved in complex feeding and emotional behaviors [63,64]. Importantly, GDF15's effects on body weight are independent of other well—known pathways that impact feeding such as melanocortin-4 and leptin receptors as well as classic incretin pathway glucagon-like peptide-1 (GLP-1) [24]. Tsai et al. [65] demonstrated that GDF15's serum levels vary in a diurnal pattern, similar to that of leptin. They also stated that very subtle postprandial changes in GDF15's serum levels were observed and suggested that GDF15 does not demonstrate strong satiety-promoting effects, but rather acts as a long-term regulator of energy homeostasis [65].

In summary, several lines of evidence suggest that GDF15-GFRAL-RET is a central neuronal pathway regulating feeding and behaviors associated with feeding, such as food aversion.

4. HUMAN GENETICS OF GDF15 AND GFRAL

Several human genetic studies have implicated GDF15 or GFRAL in obesity with body mass index (BMI) or other relevant diseases related to feeding or nausea. In 2015, genetic BMI studies identified 97 genome-wide significant loci, with one associated with GDF15 [66]. In another study, GDF15's level was shown to be inversely related to BMI in a non-obese monozygotic twin pair, with one twin having a higher serum GDF15 level and a lower BMI than his/her identical sibling [65]. That same study demonstrated that circulating concentrations of GDF15 varied in a diurnal pattern, suggesting that it may also have a physiological role in the regulation of energy homeostasis in humans [65]. GDF15 is elevated in obese patients compared with non-obese individuals [28,67,68]. Increased levels of circulating GDF15 in obese patients may not be the driving mechanism for obesity but rather

reflect a compensatory mechanism that tries to limit energy intake when it is pursued in excess. However, the human genetic signal to demonstrate that the GDF15/GFRAL axis is associated with BMI is not very strong because in another study, no relationship of GDF15 was observed with any anthropometric measurements (BMI, waist-hip ratio, waist circumference, whole-body lean mass, type 2 diabetes, fasting glucose, glycated hemoglobin, fasting insulin, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, total cholesterol, triglycerides, and coronary artery disease) [69]. These authors concluded that lifelong GDF15 levels are not causally associated with body weight, glycemic phenotypes, lipids, or coronary artery disease in humans [69]. Very few studies have investigated the functional consequences of GDF15's locus association with obesity. Moon et al. [70] performed a mendelian randomization through a single nucleotide polymorphism rs7226, which is significantly associated with GDF15 expression in the whole blood and has been linked to the GDF15 concentration [71]. In their study, 33 traits related to obesity and 27 traits related to the concentration of leukocytes in the blood obtained from a variety of publicly available human genome-wide association study datasets were assessed. Increased GDF15 expression was significantly associated with reduced obesity and fat mass for 24 traits [70].

Studies of pregnancy-related vomiting in humans suggest that placentation, appetite, and GDF15 are linked to hyperemesis gravidarum. In these studies, maternal GDF15 levels were significantly associated with pregnancy-related vomiting [72]; at week 15 of pregnancy, serum GDF15 concentrations were positively associated with second trimester vomiting and maternal anti-emetic use in pregnancy. This was further confirmed by Fejzo et al. [73], whose studies of hyperemesis gravidarum demonstrated that one of the most significantly associated loci on chromosome 19p13.11 contained genes associated with GDF15. In addition, the gene encoding the receptor for GDF15, the brainstem-restricted receptor GFRAL, was associated with nausea and vomiting in pregnancy, further supporting a previously unknown biological connection between GDF15 and hyperemesis gravidarum [73]. The same group subsequently published a study that showed variants associated with altered GDF15 levels segregate with disease in hyperemesis gravidarum families, a finding that may explain familial susceptibility to hyperemesis gravidarum [40]. These early human genetic findings highlight the relationship between BMI, potential nausea, and hyperemesis gravidarum.

5. MOUSE GENETICS OF GDF15 AND GFRAL

Ablation of GDF15 or GFRAL in genetically engineered mice results in increased body weight [17,18,24,25]. In one study, body weight of adult female GDF15-deficient mice was significantly increased by 16% [18]. Another laboratory study found that male GDF15$^{-/-}$ mice were 6% heavier than their age-matched male GDF15$^{+/+}$ control mice, and female GDF15$^{-/-}$ mice weighed on average 10% more than female controls [17]. Interestingly, the females (but not the males) showed an increase in food intake on normal chow and reduced energy expenditure compared with controls [17]. When fed a high-fat diet (HFD), another study reported that males (but not females) showed a significant increase in body weight and reduced motor activity compared with their counterpart wild-type mice [74]. The discrepancy in metabolic rates of GDF15 knockout mice measured in different laboratories may be explained by the fact that the phenotype of these mice is not very robust. A slight difference in one of the parameters (mouse strain, diet, or stress levels in the laboratory) may have uncovered a slight discrepancy in the measurement of the metabolic rate between these

laboratories. Metformin treatment in HFD-fed mice has been shown to significantly increase the metabolic rate, which was blocked by GFRAL antagonism [75]. The author of this manuscript concluded that under conditions in which GDF15 levels are increased by metformin, body weight reduction is contributed by both reduced food intake and high energy expenditure [75]. It was also reported that when GFRAL knockout mice were fed a normal chow diet, they were no different than their wild-type littermates in terms of food intake, energy expenditure, or body weight [24]. However, when these knockout mice were fed a HFD, their phenotype became more evident: they gained more weight and became glucose-intolerant compared with their wild-type counterparts [24]. When fed the HFD for 12 weeks, weight increased in the GFRAL knockout mice, with a greater increase in fat mass (32%) than lean mass (9%) [24]. Another group confirmed these findings, reporting that GFRAL knockout mice fed a HFD for 18 weeks gained ∼ 35% of fat mass [25]. This increase was attributed to a slight increase in food intake [25]. Interestingly, it was reported that neither GDF15 nor GFRAL knockout mice showed significant differences in their relative food choices compared with their wild-type controls [76]. This latter finding indicates that endogenous GDF15 does not play a major role in food choice in this paradigm.

Several studies have evaluated GDF15 overexpression in mice. Transgenic mice from two independent laboratories that overexpressed GDF15 developed lower body weight and fat mass than their non-transgenic controls [20] and no differences were observed in food consumption. In 2012, Macia et al. demonstrated that long-term overexpression of GDF15 decreased body weight, decreased fat mass, and improved glucose homeostasis under normal chow feeding conditions or when fed a HFD [19]. No data with GFRAL overexpression in mice have been published. While many of the physiological effects of GDF15 remain unclear, these aggregated findings support that, in mice, GDF15 is a regulator of body weight.

6. RECENT DEVELOPMENTS OF GDF15

Aside from the recent identification of the endogenous receptor system for GDF15, new developments in GDF15-related research include evidence about the possible mechanism of action of GDF15. For example, GDF15 may play a protective role in inflammation resulting from pathogenic infection and sepsis [77]. This assertion is supported by evidence showing that blocking GDF15 activity using a neutralizing antibody resulted in reduced death in rodent sepsis models [77]. Neurons activated by GDF15-GFRAL interactions in the AP induce catecholamine-mediated signals to the liver, inducing triglyceride metabolism and mobilizing lipids as a tissue energy source [77]. These findings have important implications for disease states with inflammation as a driver of morbidity and mortality [78]. The concept of a neural–immune interaction initiated as a result of a pathogenic insult is not new. It has been shown that activation of vagus afferent nerve fibers by endotoxins or cytokines induce anti-inflammatory responses [79,80]. Stimulation of the vagus nerve can also reduce an endotoxin-induced inflammatory response [81]. In rats treated with endotoxin, stimulation of the vagus nerve led to increases in anti-inflammatory cytokines, whereas vagotomy led to increases in pro-inflammatory cytokines [82]. These data show that GDF15 activation of AP neurons, followed by vagal activation, has effects beyond weight regulation and feeding behavior and GDF15 may be an important component of an anti-inflammatory milieu.

In another recent area of research, Borner et al. demonstrated that GDF15 showed short-term effects on reducing food intake and longer-term effects on conditioned food aversion [83]. When rats were

administered GDF15 and then exposed to a food substance, they displayed avoidance and disgust toward the food, which lasted well after the anorectic effect of GDF15 had waned; the food aversion remained in the absence of effects on gastric emptying [83]. These findings suggest that a long-term taste aversion has been established, presumably through involvement of the CTZ as previously discussed. Observations such as these provide strong evidence that the GFRAL receptor located in the AP drives the physiological action of GDF15.

7. GDF15 FOR TREATING OBESITY

7.1. GDF15 in preclinical obesity models

Recombinant GDF15 or extended half-life GDF15 molecules have been administered in multiple models of obesity in mice, rats, and monkeys [24,25,28,56,84,85]. In all the preclinical models studied, administration of various GDF15 molecules consistently resulted in decreased food intake and body weight. For example, in C57Bl6 mice, GDF15 treatment resulted in a dose-dependent reduction in food intake that reached statistical significance at the highest dose (0.1 mg/kg) [86]. In a separate study with C57Bl6 mice, administration of either mouse or human GDF15 lowered 6-hour food intake, and this effect was achieved through the suppression of meal size rather than meal frequency [25]. Also in C57Bl6 mice, GDF15 treatment at doses of 0.01 mg/kg and 0.1 mg/kg reduced saccharin consumption and increased water consumption compared with a vehicle-treated control [86]. These data demonstrate that acute administration of GDF15 can elicit an aversive response in rodents. Along the same lines, Frikke et al. [76] found that GDF15 treatment significantly lowered the preference for fat intake in mice, findings that confirmed the data previously described by Xiong et al. [28]. The same authors demonstrated that recombinant GDF15 treatments reduced food intake and body weight and improved metabolic profiles in mice, rats, and obese cynomolgus monkeys [28]. In other preclinical models of diabetes/obesity, including ob/ob, db/db, and KKAy mice, overexpression of murine or human GDF15 using an adeno-associated virus (AAV) system improved body weight and metabolic parameters. Both recombinant murine (rm) GDF15 and recombinant human (rh) GDF15 proteins demonstrated very similar efficacy and potency in reducing food intake in ob/ob mice [28]. In these mice, treatment with rhGDF15 protein reduced food intake and body weight. In diet-induced obese (DIO) mice, rhGDF15 protein treatment decreased food intake, body weight, blood glucose, serum insulin, serum triglyceride, and cholesterol concentrations and improved glucose tolerance in a dose-dependent manner [28]. At the highest concentration tested, rhGDF15 proteins (at 10 mmol/kg) decreased food intake by $\sim 80\%$ (compared to food intake of vehicle-treated animals) and body weight by $\sim 20\%$ compared to baseline [28]. To determine whether reduced caloric intake was responsible for the weight loss observed with GDF15 treatment, Mullican et al. restricted caloric intake in vehicle-treated DIO mice to match the amount of food ingested by GDF15-treated DIO mice [25]. Because the same amount of weight loss was observed in these calorie-restricted, vehicle-treated mice vs the GDF15-treated mice, the authors concluded that GDF15-induced weight loss in this disease model was primarily due to decreased energy intake [25].

Similar to the previously discussed findings in mice [25], daily treatment with rhGDF15 in obese cynomolgus monkeys reduced food intake, body weight, plasma insulin, and plasma triglyceride concentrations and improved glucose tolerance [25]. Other studies have used long-acting forms of the GDF15 protein (administered weekly instead of daily) and replicated the effects observed with recombinant protein in multiple species, including obese cynomolgus monkeys [28]. Another long-acting GDF15 molecule, human serum albumin (HSA) GDF15, at a dose of 10 nmol/kg when administered weekly to obese cynomolgus monkeys showed significantly less daily food intake than vehicle-treated animals [25]. In addition, significant weight loss was observed in monkeys treated with 3 or 10 nmol/kg [25].

Agonistic GDF15 pharmacological approaches that have been tested by multiple independent laboratories previously listed have demonstrated consistent effects on food intake and weight loss, taste aversion, and improving multiple metabolic parameters. Interestingly, antagonistic approaches have demonstrated the expected opposite effects when treating mice with a monoclonal antibody to GDF15 shortly after initiating feeding an HFD; these mice showed a more rapid gain of body weight and adiposity than control groups that did not receive the antibody [84]. Consistent with the GDF15 Abs, mAb26, mAb1, and mAb2 inhibition studies [28,84], disruption of GDF15's actions by knockdown of GFRAL with AAV short hairpin RNA increased body weight and adiposity in HFD-fed mice [84]. Another group recently reported that an antagonistic monoclonal antibody, 3P10, designed to prevent GDF15-driven interaction of RET with GFRAL, reversed lipid oxidation in tumor-bearing mice [87]. They demonstrated that activation of the GFRAL/RET pathway induced expression of genes involved in lipid metabolism in adipose tissues independent of anorexia, leading to reduced adipose and muscle mass in tumor-bearing mice [87].

7.2. GDF15 molecules being developed and tested for the treatment of obesity

GDF15 has a half-life of ~ 3 h in the circulation [28], and engineering a longer half-life would make GDF15 a more patient-friendly therapy. Based on reports in the public domain, it can be assumed that several pharmaceutical companies have attempted to develop long-lasting GDF15 analogues to treat obesity and/or cachexia [23—26,28]. The two approaches are fusing GDF15 with an immunoglobulin Fc domain or HSA. Both approaches slow the renal clearance of these large molecules. For example, one group used a structure-based approach to design GDF15 fusion proteins with a longer half-life and higher production yield than native protein [28]. These Fc-GDF15 fusion proteins were tested in obese rodent and obese cynomolgus monkey models and demonstrated weight reductions in both models [28]. Another group used an HSA-GDF15 fusion molecule with an extended serum half-life [25]. Administration of the HSA-GDF15 fusion molecule to spontaneously obese cynomolgus monkeys resulted in significant weight loss after 4 weeks of exposure [25]. The weight loss effects in obese cynomolgus monkeys are encouraging, and hopefully these successful responses bode well for translation to treatment responses in obese human patients. A patent assigned to NGM Biopharmaceuticals (US patent 9,161,966, issued on October 20, 2015) contains data showing weight loss when HSA-GDF15 is administered to ob/ob mice. To date, no non-human primate data have been reported from this group. A patent application from Eli Lilly and Company (US 2019/0309033 A1, published on October 10, 2019) includes data using an Fc-GDF15 fusion protein administered by subcutaneous injection to DIO mice every 3 days for 21 days. Body weights and food intake both showed dose-dependent reductions on day 16. No non-human primate testing was described. Novo Nordisk
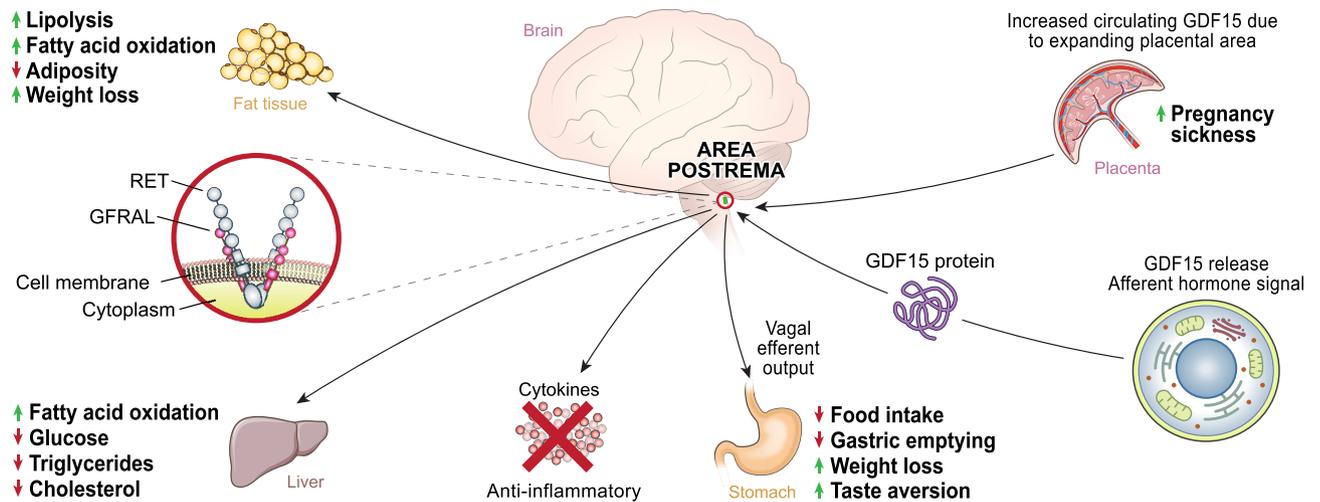
Figure 1: Illustration of the receptor complex and physiological effects of GDF15.

A/S has a published patent application (US 2020/0079829 A1, published on March 12, 2020) describing a chemically modified MIC-1 (an alias of GDF15) compound administered for 7 days to male Sprague—Dawley rats decreased both body weight and food intake. The application also describes administration of a single dose, either 1 nmol/kg or 9 nmol/kg, of GDF15 compounds to female Landrace Yorkshire Duroc pigs with body weight measurements taken 7, 14, or 21 days after dose administration. Body weights were reduced in both dose groups on all 3 days. The results observed to date in preclinical animal models are encouraging; however, entry into the clinical trial setting will confirm whether GDF15-induced weight loss effects in other species are translatable to humans.

8. CHALLENGES FOR POTENTIAL GDF15 THERAPIES

As much potential as GDF15 might have as a weight loss treatment, there will be potential safety risks when administering GDF15 analogues to humans. A central concern is the AP location of the GFRAL receptor. Because this area is thought to function as a sensor for ingested toxic/noxious substances and may trigger expulsion of such substances via nausea and vomiting, any GDF15 analogue may also have the potential to cause nausea and vomiting. This may be a particular problem with the long-lasting GDF15 analogues that would be desirable to treat obesity in humans. Indeed, analogues of GLP-1 are known to induce gastrointestinal side effects such as nausea and vomiting [88], with nausea being the most common, affecting up to 50% of patients [88]. However, nausea from GLP-1 analogues tends to be mild or moderate and diminishes as treatment continues [89,90]. Human clinical trials will determine whether administration of GDF15 analogues induces nausea and vomiting, and if so, whether the effect is transient and diminishes with continued administration.
Another potential challenge for GDF15 with long-term use as an obesity therapy that needs to be addressed is how GDF15 has been reported to be associated with cancers and all-cause mortality [15,21]. To address some of these concerns, it is interesting to highlight a publication by Xiong et al. in which the investigators used an AAV-expressing system in DIO mice, which increased the circulating human GDF15 levels by > 30 fold for a year. Clinical and histopathological analysis of 1-year-old DIO mice treated with AAV-expressed human GDF15 revealed no detrimental effects associated with GDF15 overexpression in the brain, heart, liver, gallbladder, spleen, pancreas, kidney, skeletal muscle, stomach, duodenum, jejunum, ileum, colon, prostate gland, preputial gland, skin, or adipose tissue [28], suggesting that long-term exposure to elevated GDF15 levels does not cause an increase in tumorgenicity or mortality.

9. SUMMARY/CONCLUSIONS

In summary, the biology of GDF15 is a rapidly evolving and exciting field. Interest from the broader scientific community is driven by a) emerging findings that GFRAL is the cognate receptor for GDF15, b) GFRAL's anatomic location is highly restricted to the AP, a brain region specialized as a CTZ, and c) observations that GDF15 administration triggers weight loss in preclinical animal models (Figure 1). Pharmaceutical industry efforts are underway to produce engineered GDF15 molecules that are efficacious, safe, and have longer half-lives in the blood than native GDF15 protein. Molecules that have good pharmacokinetic and pharmacodynamic properties and the potential for safe administration in patients could become first-in-human candidates. If human clinical trials are successful, and nausea and vomiting are manageable side effects, then GDF15 analogues could be a cutting-edge weight loss therapy.

CONFLICT OF INTEREST

The authors are employees of and hold stock in Amgen Inc.

AUTHOR CONTRIBUTIONS

C.H. and M.M.V.: Conceptualization, original draft preparation, writing, and editing.

REFERENCES

[1] Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C., et al., 2014. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 384(9945):766—781.

[2] Wirth, A., Wabitsch, M., Hauner, H., 2014. The prevention and treatment of obesity. Dtsch Arztebl Int 111(42):705—713.

[3] Long, S.D., O'Brien, K., MacDonald Jr., K.G., Leggett-Frazier, N., Swanson, M.S., Pories, W.J., et al., 1994. Weight loss in severely obese subjects prevents the progression of impaired glucose tolerance to type II diabetes. A longitudinal interventional study. Diabetes Care 17(5):372—375.

[4] Wong, D., Sullivan, K., Heap, G., 2012. The pharmaceutical market for obesity therapies. Nature Reviews Drug Discovery 11(9):669—670.

[5] Bray, G.A., Greenway, F.L., 2007. Pharmacological treatment of the overweight patient. Pharmacological Reviews 59(2):151—184.

[6] Bootcov, M.R., Bauskin, A.R., Valenzuela, S.M., Moore, A.G., Bansal, M., He, X.Y., et al., 1997. MIC-1, a novel macrophage inhibitory cytokine, is a divergent member of the TGF-beta superfamily. Proceedings of the National Academy of Sciences of the United States of America 94(21):11514—11519.

[7] Hromas, R., Hufford, M., Sutton, J., Xu, D., Li, Y., Lu, L., 1997. PLAB, a novel placental bone morphogenetic protein. Biochimica et Biophysica Acta 1354(1):40—44.

[8] Lawton, L.N., Bonaldo, M.F., Jelenc, P.C., Qiu, L., Baumes, S.A., Marcelino, R.A., et al., 1997. Identification of a novel member of the TGF-beta superfamily highly expressed in human placenta. Gene 203(1):17—26.

[9] Paralkar, V.M., Vail, A.L., Grasser, W.A., Brown, T.A., Xu, H., Vukicevic, S., et al., 1998. Cloning and characterization of a novel member of the transforming growth factor-beta/bone morphogenetic protein family. Journal of Biological Chemistry 273(22):13760—13767.

[10] Daopin, S., Piez, K.A., Ogawa, Y., Davies, D.R., 1992. Crystal structure of transforming growth factor-beta 2: an unusual fold for the superfamily. Science 257(5068):369—373.

[11] Schlunegger, M.P., Grutter, M.G., 1992. An unusual feature revealed by the crystal structure at 2.2 A resolution of human transforming growth factor-beta 2. Nature 358(6385):430—434.

[12] Fairlie, W.D., Moore, A.G., Bauskin, A.R., Russell, P.K., Zhang, H.P., Breit, S.N., 1999. MIC-1 is a novel TGF-beta superfamily cytokine associated with macrophage activation. Journal of Leukocyte Biology 65(1):2—5.

[13] Unsicker, K., Spittau, B., Krieglstein, K., 2013. The multiple facets of the TGF-beta family cytokine growth/differentiation factor-15/macrophage inhibitory cytokine-1. Cytokine & Growth Factor Reviews 24(4):373—384.

[14] Corre, J., Hebraud, B., Bourin, P., 2013. Concise review: growth differentiation factor 15 in pathology: a clinical role? Stem Cells Transl Med 2(12):946—952.

[15] Breit, S.N., Johnen, H., Cook, A.D., Tsai, V.W., Mohammad, M.G., Kuffner, T., et al., 2011. The TGF-beta superfamily cytokine, MIC-1/GDF15: a pleotrophic cytokine with roles in inflammation, cancer and metabolism. Growth Factors 29(5):187—195.

[16] Kempf, T., Zarbock, A., Widera, C., Butz, S., Stadtmann, A., Rossaint, J., et al., 2011. GDF-15 is an inhibitor of leukocyte integrin activation required for survival after myocardial infarction in mice. Nature Medicine 17(5):581—588.

[17] Tsai, V.W., Macia, L., Johnen, H., Kuffner, T., Manadhar, R., Jorgensen, S.B., et al., 2013. TGF-b superfamily cytokine MIC-1/GDF15 is a physiological appetite and body weight regulator. PloS One 8(2):e55174.

[18] Strelau, J., Strzelczyk, A., Rusu, P., Bendner, G., Wiese, S., Diella, F., et al., 2009. Progressive postnatal motoneuron loss in mice lacking GDF-15. Journal of Neuroscience 29(43):13640—13648.

[19] Macia, L., Tsai, V.W., Nguyen, A.D., Johnen, H., Kuffner, T., Shi, Y.C., et al., 2012. 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(4):e34868.

[20] Baek, S.J., Okazaki, R., Lee, S.H., Martinez, J., Kim, J.S., Yamaguchi, K., et al., 2006. Nonsteroidal anti-inflammatory drug-activated gene-1 over expression in transgenic mice suppresses intestinal neoplasia. Gastroenterology 131(5):1553—1560.

[21] Wiklund, F.E., Bennet, A.M., Magnusson, P.K., Eriksson, U.K., Lindmark, F., Wu, L., et al., 2010. Macrophage inhibitory cytokine-1 (MIC-1/GDF15): a new marker of all-cause mortality. Aging Cell 9(6):1057—1064.

[22] Olsen, O.E., Skjaervik, A., Stordal, B.F., Sundan, A., Holien, T., 2017. TGF-beta contamination of purified recombinant GDF15. PloS One 12(11):e0187349.

[23] Emmerson, P.J., Wang, F., Du, Y., Liu, Q., Pickard, R.T., Gonciarz, M.D., et al., 2017. The metabolic effects of GDF15 are mediated by the orphan receptor GFRAL. Nature Medicine 23(10):1215—1219.

[24] Hsu, J.Y., Crawley, S., Chen, M., Ayupova, D.A., Lindhout, D.A., Higbee, J., et al., 2017. Non-homeostatic body weight regulation through a brainstem-restricted receptor for GDF15. Nature 550(7675):255—259.

[25] Mullican, S.E., Lin-Schmidt, X., Chin, C.N., Chavez, J.A., Furman, J.L., Armstrong, A.A., et al., 2017. GFRAL is the receptor for GDF15 and the ligand promotes weight loss in mice and nonhuman primates. Nature Medicine 23(10):1150—1157.

[26] Yang, L., Chang, C.C., Sun, Z., Madsen, D., Zhu, H., Padkjaer, S.B., et al., 2017. GFRAL is the receptor for GDF15 and is required for the anti-obesity effects of the ligand. Nature Medicine 23(10):1158—1166.

[27] Tsai, V.W., Manandhar, R., Jorgensen, S.B., Lee-Ng, K.K., Zhang, H.P., Marquis, C.P., et al., 2014. 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(6):e100370.

[28] Xiong, Y., Walker, K., Min, X., Hale, C., Tran, T., Komorowski, R., et al., 2017. Long-acting MIC-1/GDF15 molecules to treat obesity: evidence from mice to monkeys. Science Translational Medicine 9(412).

[29] Baek, S.J., Eling, T., 2019. Growth differentiation factor 15 (GDF15): a survival protein with therapeutic potential in metabolic diseases. Pharmacology & Therapeutics 198:46—58.

[30] Tsai, V.W.W., Husaini, Y., Sainsbury, A., Brown, D.A., Breit, S.N., 2018. The MIC-1/GDF15-GFRAL pathway in energy homeostasis: implications for obesity, cachexia, and other associated diseases. Cell Metabolism 28(3):353—368.

[31] Baek, S.J., Kim, K.S., Nixon, J.B., Wilson, L.C., Eling, T.E., 2001. Cyclooxygenase inhibitors regulate the expression of a TGF-beta superfamily member that has proapoptotic and antitumorigenic activities. Molecular Pharmacology 59(4):901—908.

[32] Li, P.X., Wong, J., Ayed, A., Ngo, D., Brade, A.M., Arrowsmith, C., et al., 2000. Placental transforming growth factor-beta is a downstream mediator of the growth arrest and apoptotic response of tumor cells to DNA damage and p53 overexpression. Journal of Biological Chemistry 275(26):20127—20135.

[33] Hsiao, E.C., Koniaris, L.G., Zimmers-Koniaris, T., Sebald, S.M., Huynh, T.V., Lee, S.J., 2000. Characterization of growth-differentiation factor 15, a transforming growth factor beta superfamily member induced following liver injury. Molecular and Cellular Biology 20(10):3742—3751.

[34] Bauskin, A.R., Brown, D.A., Junankar, S., Rasiah, K.K., Eggleton, S., Hunter, M., et al., 2005. The propeptide mediates formation of stromal stores of PROMIC-1: role in determining prostate cancer outcome. Cancer Research 65(6):2330—2336.

[35] Brown, D.A., Ward, R.L., Buckhaults, P., Liu, T., Romans, K.E., Hawkins, N.J., et al., 2003. MIC-1 serum level and genotype: associations with progress and prognosis of colorectal carcinoma. Clinical Cancer Research 9(7):2642—2650.

[36] Breit, S.N., Carrero, J.J., Tsai, V.W., Yagoutifam, N., Luo, W., Kuffner, T., et al., 2012. Macrophage inhibitory cytokine-1 (MIC-1/GDF15) and mortality in end-stage renal disease. Nephrology Dialysis Transplantation 27(1):70—75.

[37] Welsh, J.B., Sapinoso, L.M., Kern, S.G., Brown, D.A., Liu, T., Bauskin, A.R., et al., 2003. Large-scale delineation of secreted protein biomarkers overexpressed in cancer tissue and serum. Proceedings of the National Academy of Sciences of the United States of America 100(6):3410—3415.

[38] Wollert, K.C., Kempf, T., Peter, T., Olofsson, S., James, S., Johnston, N., et al., 2007. Prognostic value of growth-differentiation factor-15 in patients with non-ST-elevation acute coronary syndrome. Circulation 115(8):962—971.

[39] Moore, A.G., Brown, D.A., Fairlie, W.D., Bauskin, A.R., Brown, P.K., Munier, M.L., et al., 2000. The transforming growth factor-ss superfamily cytokine macrophage inhibitory cytokine-1 is present in high concentrations in the serum of pregnant women. Journal of Clinical Endocrinology & Metabolism 85(12):4781—4788.

[40] Fejzo, M.S., Arzy, D., Tian, R., MacGibbon, K.W., Mullin, P.M., 2018. Evidence GDF15 plays a role in familial and recurrent hyperemesis gravidarum. Geburtshilfe und Frauenheilkunde 78(9):866—870.

[41] Tong, S., Marjono, B., Brown, D.A., Mulvey, S., Breit, S.N., Manuelpillai, U., et al., 2004. Serum concentrations of macrophage inhibitory cytokine 1 (MIC 1) as a predictor of miscarriage. Lancet 363(9403):129—130.

[42] Tong, S., Ngian, G.L., Onwude, J.L., Permezel, M., Saglam, B., Hay, S., et al., 2012. Diagnostic accuracy of maternal serum macrophage inhibitory cytokine-1 and pregnancy-associated plasma protein-A at 6-10 weeks of gestation to predict miscarriage. Obstetrics & Gynecology 119(5):1000—1008.

[43] Jing, S., Wen, D., Yu, Y., Holst, P.L., Luo, Y., Fang, M., et al., 1996. GDNF-induced activation of the ret protein tyrosine kinase is mediated by GDNFR-alpha, a novel receptor for GDNF. Cell 85(7):1113—1124.

[44] Hofstra, R.M., Landsvater, R.M., Ceccherini, I., Stulp, R.P., Stelwagen, T., Luo, Y., et al., 1994. A mutation in the RET proto-oncogene associated with multiple endocrine neoplasia type 2B and sporadic medullary thyroid carcinoma. Nature 367(6461):375—376.

[45] Ikeda, I., Ishizaka, Y., Tahira, T., Suzuki, T., Onda, M., Sugimura, T., et al., 1990. Specific expression of the ret proto-oncogene in human neuroblastoma cell lines. Oncogene 5(9):1291—1296.

[46] Iwamoto, T., Taniguchi, M., Asai, N., Ohkusu, K., Nakashima, I., Takahashi, M., 1993. cDNA cloning of mouse ret proto-oncogene and its sequence similarity to the cadherin superfamily. Oncogene 8(4):1087—1091.

[47] Li, Z., Wang, B., Wu, X., Cheng, S.Y., Paraoan, L., Zhou, J., 2005. Identification, expression and functional characterization of the GRAL gene. Journal of Neurochemistry 95(2):361—376.

[48] Wilson, J.G., Scott, W.J., Ritter, E.J., Fradkin, R., 1975. Comparative distribution and embryotoxicity of hydroxyurea in pregnant rats and rhesus monkeys. Teratology 11(2):169—178.

[49] Borison, H.L., Wang, S.C., 1953. Physiology and pharmacology of vomiting. Pharmacological Reviews 5(2):193—230.

[50] Borison, H.L., Borison, R., McCarthy, L.E., 1984. Role of the area postrema in vomiting and related functions. Federation Proceedings 43(15):2955—2958.

[51] Borison, H.L., 1989. Area postrema: chemoreceptor circumventricular organ of the medulla oblongata. Progress in Neurobiology 32(5):351—390.

[52] Kalia, M., Mesulam, M.M., 1980. Brain stem projections of sensory and motor components of the vagus complex in the cat: II. Laryngeal, tracheobronchial, pulmonary, cardiac, and gastrointestinal branches. Journal of Comparative Neurology 193(2):467—508.

[53] Kalia, M., Welles, R.V., 1980. Brain stem projections of the aortic nerve in the cat: a study using tetramethyl benzidine as the substrate for horseradish peroxidase. Brain Research 188(1):23—32.

[54] Opsahl, C.A., Powley, T.L., 1977. Body weight and gastric acid secretion in rats with subdiaphragmatic vagotomy and lateral hypothalamic lesions. Journal of Comparative & Physiological Psychology 91(6):1284—1296.

[55] Rezek, M., Vanderweele, D.A., Novin, D., 1975. Stages in the recovery of feeding following vagotomy in rabbits. Behavioral Biology 14(1):75—84.

[56] Johnen, H., Lin, S., Kuffner, T., Brown, D.A., Tsai, V.W., Bauskin, A.R., et al., 2007. Tumor-induced anorexia and weight loss are mediated by the TGF-beta superfamily cytokine MIC-1. Nature Medicine 13(11):1333—1340.

[57] Schwartz, G.J., 2010. Brainstem integrative function in the central nervous system control of food intake. Forum of nutrition 63:141—151.

[58] Bernstein, I.L., Treneer, C.M., Kott, J.N., 1985. Area postrema mediates tumor effects on food intake, body weight, and learned aversions. American Journal of Physiology 249(3 Pt 2):R296—R300.

[59] Wollert, K.C., Kempf, T., Lagerqvist, B., Lindahl, B., Olofsson, S., Allhoff, T., et al., 2007. Growth differentiation factor 15 for risk stratification and selection of an invasive treatment strategy in non ST-elevation acute coronary syndrome. Circulation 116(14):1540—1548.

[60] LeDoux, J., 2007. The amygdala. Current Biology 17(20):R868—R874.

[61] LeDoux, J.E., 2000. Emotion circuits in the brain. Annual Review of Neuroscience 23:155—184.

[62] King, B.M., Rossiter, K.N., Stines, S.G., Zaharan, G.M., Cook, J.T., Humphries, M.D., et al., 1998. Amygdaloid-lesion hyperphagia: impaired response to caloric challenges and altered macronutrient selection. American Journal of Physiology 275(2):R485—R493.

[63] Ahn, S., Phillips, A.G., 2002. Modulation by central and basolateral amygdalar nuclei of dopaminergic correlates of feeding to satiety in the rat nucleus accumbens and medial prefrontal cortex. Journal of Neuroscience 22(24): 10958—10965.

[64] Carlini, V.P., Varas, M.M., Cragnolini, A.B., Schioth, H.B., Scimonelli, T.N., de Barioglio, S.R., 2004. Differential role of the hippocampus, amygdala, and dorsal raphe nucleus in regulating feeding, memory, and anxiety-like behavioral responses to ghrelin. Biochemical and Biophysical Research Communications 313(3):635—641.

[65] Tsai, V.W., Macia, L., Feinle-Bisset, C., Manandhar, R., Astrup, A., Raben, A., et al., 2015. 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(7):e0133362.

[66] Locke, A.E., Kahali, B., Berndt, S.I., Justice, A.E., Pers, T.H., Day, F.R., et al., 2015. Genetic studies of body mass index yield new insights for obesity biology. Nature 518(7538):197—206.

[67] Kempf, T., Guba-Quint, A., Torgerson, J., Magnone, M.C., Haefliger, C., Bobadilla, M., et al., 2012. Growth differentiation factor 15 predicts future insulin resistance and impaired glucose control in obese nondiabetic individuals: results from the XENDOS trial. European Journal of Endocrinology 167(5):671—678.

[68] Vila, G., Riedl, M., Anderwald, C., Resl, M., Handisurya, A., Clodi, M., et al., 2011. The relationship between insulin resistance and the cardiovascular biomarker growth differentiation factor-15 in obese patients. Clinical Chemistry 57(2):309—316.

[69] Cheung, C.L., Tan, K.C.B., Au, P.C.M., Li, G.H.Y., Cheung, B.M.Y., 2019. Evaluation of GDF15 as a therapeutic target of cardiometabolic diseases in human: a Mendelian randomization study. EBioMedicine 41:85—90.

[70] Moon, J.S., Goeminne, L.J.E., Kim, J.T., Tian, J.W., Kim, S.H., Nga, H.T., et al., 2020. Growth differentiation factor 15 protects against the aging-mediated systemic inflammatory response in humans and mice. Aging Cell 19(8):e13195.

[71] Jiang, J., Thalamuthu, A., Ho, J.E., Mahajan, A., Ek, W.E., Brown, D.A., et al., 2018. A meta-analysis of genome-wide association studies of growth differentiation factor-15 concentration in blood. Frontiers in Genetics 9:97.

[72] Petry, C.J., Ong, K.K., Burling, K.A., Barker, P., Goodburn, S.F., Perry, J.R.B., et al., 2018. 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.

[73] Fejzo, M.S., Sazonova, O.V., Sathirapongsasuti, J.F., Hallgrimsdottir, I.B., Vacic, V., MacGibbon, K.W., et al., 2018. Placenta and appetite genes GDF15

and IGFBP7 are associated with hyperemesis gravidarum. Nature Communications 9(1):1178.

[74] Tran, T., Yang, J., Gardner, J., Xiong, Y., 2018. GDF15 deficiency promotes high fat diet-induced obesity in mice. PloS One 13(8):e0201584.

[75] Coll, A.P., Chen, M., Taskar, P., Rimmington, D., Patel, S., Tadross, J.A., et al., 2020. GDF15 mediates the effects of metformin on body weight and energy balance. Nature 578(7795):444—448.

[76] Frikke-Schmidt, H., Hultman, K., Galaske, J.W., Jorgensen, S.B., Myers Jr., M.G., Seeley, R.J., 2019. GDF15 acts synergistically with liraglutide but is not necessary for the weight loss induced by bariatric surgery in mice. Mol Metab 21:13—21.

[77] Luan, H.H., Wang, A., Hilliard, B.K., Carvalho, F., Rosen, C.E., Ahasic, A.M., et al., 2019. GDF15 is an inflammation-induced central mediator of tissue tolerance. Cell 178(5):1231—1244 e1211.

[78] Anrather, J., Iadecola, C., 2016. Inflammation and stroke: an overview. Neurotherapeutics 13(4):661—670.

[79] Sternberg, E.M., 1997. Neural-immune interactions in health and disease. Journal of Clinical Investigation 100(11):2641—2647.

[80] Watkins, L.R., Maier, S.F., 1999. Implications of immune-to-brain communication for sickness and pain. Proceedings of the National Academy of Sciences of the United States of America 96(14):7710—7713.

[81] Borovikova, L.V., Ivanova, S., Zhang, M., Yang, H., Botchkina, G.I., Watkins, L.R., et al., 2000. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature 405(6785):458—462.

[82] Mihaylova, S., Killian, A., Mayer, K., Pullamsetti, S.S., Schermuly, R., Rosengarten, B., 2012. Effects of anti-inflammatory vagus nerve stimulation on the cerebral microcirculation in endotoxinemic rats. Journal of Neuro-inflammation 9:183.

[83] Borner, T., Wald, H.S., Ghidewon, M.Y., Zhang, B., Wu, Z., De Jonghe, B.C., et al., 2020. GDF15 induces an aversive visceral malaise state that drives anorexia and weight loss. Cell Reports 31(3):107543.

[84] Tsai, V.W., Lin, S., Brown, D.A., Salis, A., Breit, S.N., 2016. Anorexia-cachexia and obesity treatment may be two sides of the same coin: role of the TGF-b superfamily cytokine MIC-1/GDF15. International Journal of Obesity 40(2):193—197.

[85] Hinke, S.A., 2000. Growth Differentiation Factor-15 (GDF-15) inhibits gastric emptying in rodents as part of its anorectic mechanism of action. Diabetes 67.283-LB.

[86] Patel, S., Alvarez-Guaita, A., Melvin, A., Rimmington, D., Dattilo, A., Miedzybrodzka, E.L., et al., 2019. GDF15 provides an endocrine signal of nutritional stress in mice and humans. Cell Metabolism 29(3):707—718 e708.

[87] Suriben, R., Chen, M., Higbee, J., Oeffinger, J., Ventura, R., Li, B., et al., 2020. Antibody-mediated inhibition of GDF15-GFRAL activity reverses cancer cachexia in mice. Nature Medicine.

[88] Filippatos, T.D., Panagiotopoulou, T.V., Elisaf, M.S., 2014. Adverse effects of GLP-1 receptor agonists. The Review of Diabetic Studies 11(3—4):202—230.

[89] Buse, J.B., Klonoff, D.C., Nielsen, L.L., Guan, X., Bowlus, C.L., Holcombe, J.H., et al., 2007. Metabolic effects of two years of exenatide treatment on diabetes, obesity, and hepatic biomarkers in patients with type 2 diabetes: an interim analysis of data from the open-label, uncontrolled extension of three double-blind, placebo-controlled trials. Clinical Therapeutics 29(1):139—153.

[90] Ratner, R.E., Maggs, D., Nielsen, L.L., Stonehouse, A.H., Poon, T., Zhang, B., et al., 2006. Long-term effects of exenatide therapy over 82 weeks on gly-caemic control and weight in over-weight metformin-treated patients with type 2 diabetes mellitus. Diabetes, Obesity and Metabolism 8(4):419—428.