Weight gain and obesity are global health concerns contributing to morbidity with increased risks of cardiovascular disease, diabetes, liver steatohepatitis and cancer. Pharmacological therapies or bariatric surgery are often required for those who fail to adhere to diet and lifestyle modifications. Metformin, a widely used antidiabetic agent, seems to have a health benefit beyond its anti-hyperglycemic properties, with few side effects. Emerging evidence shows weight loss to be associated with metformin in both diabetic and non-diabetic individuals. Recently, the growth differentiation factor 15 (GDF-15), a member of the transforming growth factor beta superfamily, has been identified as a key mediator of metformin-induced weight loss. Metformin increases the secretion of GDF-15, which binds exclusively to glial cell-derived neurotrophic factor family receptor alpha-like (GFRAL). This gut-brain cytokine works as a prominent player in reducing food intake and body weight in health and disease, like anorexia nervosa and cancer. Herein, we critically review advances in the understanding of the weight-reducing effects of metformin via the GDF-15 pathway.

Keywords: metformin, body weight, obesity, GDF-15, GFRAL, diabetes

INTRODUCTION

Weight gain and obesity represent the second most common causes of preventable mortalities worldwide, and promote the development of cardiovascular disease, hypertension, stroke, dyslipidemia, metabolic syndrome, liver steatohepatitis, and cancer (1, 2). Diet and lifestyle modifications are the first line interventions for the management of body weight, however long-term adherence remains difficult. As such, pharmacological interventions are needed. However, due to the cost and the risk of side effects, few drugs are currently available.

Isolated from French lilac in 1920s, metformin (dimethylbiguanide) has been widely used as a first-line treatment for type 2 diabetes mellitus (DM2), owing to its excellent tolerability, safety profile, and lack of hypoglycemic effect (3–6). Aside from its anti-hyperglycemic effects, metformin...
has an additional benefit for conditions like polycystic ovary syndrome, atherosclerosis, cancer, coronavirus disease 2019 (COVID-19), and obesity (7–14). Metformin was also reported to extend lifespan in some animal models, acting as a diet mimetic agent (15–17). In contrast to other antidiabetic drugs including insulin, metformin can lead independently to glycemic control and weight loss by decreasing food intake in both diabetic and non-diabetic individuals (18–20). Thus, these observations have driven metformin’s emergence as a research priority to counteract diseases associated with obesity and aging, evoking an immune-metabolic effect.

Bodyweight is usually maintained through central nervous system (CNS) circuitry integrating peripheral metabolic feedback signals of either energy surplus or deficit (21, 22). Studies have shown converging evidence that growth differentiation factor 15 (GDF-15) mediates metformin’s effect on the gut-nervous system axis to decrease body weight (23–26). GDF-15, a member of the transforming growth factor beta (TGF-β) superfamily, has many names indicating its multiple functions, including macrophage inhibitory cytokine (MIC)-1, non-steroidal anti-inflammatory drug-inducible gene (NAG)-1, placental TGF-β (PTGF), prostate-derived factor (PDF), and placental bone morphogenetic protein (PLAB) (27). GDF-15 is a stress-induced protein, produced by a variety of cells under stress conditions including tissue injury, anoxia, and inflammation (28). Elevated circulating GDF-15 levels have been associated with more severe disease or higher mortality in people with DM2, insulin resistance, hemodialysis, cachexia, cardiovascular diseases, chronic obstructive pulmonary disease (COPD), venous thromboembolism (VTE), cancer, or obesity (28–34). Husebo et al. reported that high levels of GDF15 were independently associated with a higher rate of COPD exacerbations, and impaired respiratory function (34). From a prospective cohort of 27,158 adults followed over 13 years, among 12 tested biomarkers, GDF-15 and D-dimer were independently associated with the occurrence of VTE in multivariable analyses. Importantly, the association between GDF-15 levels and VTE was independent of D-dimer and von Willebrand factor, which are well-established biomarkers for thrombosis (35). Therefore, the elevation of GDF-15 levels during those conditions are believed to be a compensatory mechanism, as numerous evidences proposed GDF-15 as a biomarker rather than an inducer of these diseases. GDF-15 has also been shown to inhibit apoptosis and inflammation, and protects the heart from injury (36–38). In addition to these functions, GDF-15 and its tissue-specific brainstem receptor, the glial cell-derived neurotrophic factor (GDNF) family receptor alpha-like (GFRAL), have emerged as regulators of energy balance and body weight (39–41). Higher circulating GDF-15 levels have been proven to be significantly associated with weight loss in cancer patients (42, 43). To review the influence of the GDF-15 pathway on weight loss induced by metformin, we searched for the keywords “GDF-15”, “metformin”, “weight”, “diabetes drugs” alone, or in combination in the public databases of PubMed, Google Scholar, and ClinicalTrials.gov. As the relationship between GDF-15 and anti-diabetic drugs is new, we were able to discuss all English publications. In this review, we critically discuss advances in the understanding of the weight-reducing effects of metformin through modulation of GDF-15 levels in diabetic and non-diabetic individuals.

### Metformin Decreases Body Weight in Diabetic and Non-Diabetic Individuals

Dysglycemia is strongly associated with the development of overweight status or obesity, as most DM2 patients are overweight or obese (44–46). For diabetic patients, managing overweight or obesity is a crucial facet of diabetes management. However, the majority of antidiabetic agents, including insulin, thiazolidinediones, (TZDs) and insulin secretagogues, lead to body weight gain while controlling glycemia (47–49). Conversely, the first-line antidiabetic agent, metformin, has been shown to be able to decrease body weight (18, 19, 49–52). Kahn et al. (49) reported in a large randomized clinical trial (RCT) involving 4360 DM2 patients that participants lost a mean of 2.9 kg with metformin over a period of 5 years, while rosiglitazone and glyburide both induced weight gains of 4.8 and 1.6 kg, respectively. The Diabetes Prevention Program Research Group reported that metformin users had significantly reduced body weight and waist circumference compared with placebo in a 2-year RCT followed by a 8-year open-label extension. The magnitude of weight loss during the 2-year double-blind period was directly related to drug adherence, indicating a potential dose-effect (19). In 2016, a meta-analysis by Maruthur et al. showed that metformin decreased body weight more than dipeptidyl peptidase-4 (DPP-4) inhibitors which were expected to decrease body weight (18). Due to the effects of weight loss, metformin was recommended for obesity management in patients with evidence of prediabetes or insulin intolerance by AACE/ACE guidelines (53).

Aside from diabetic patients, metformin was also associated with a decrease of bodyweight in non-diabetic subjects (20, 54–57). Ejtahed et al. (20) demonstrated in a RCT that metformin induced significant weight loss compared with placebo, and this effect was associated with gut microbiota alteration in non-diabetic obese women. Furthermore, metformin has been used in obese children to promote weight loss in absence of DM2 (58–61). For adults and children with schizophrenia, metformin has been used to manage weight gain associated with anti-psychotic drugs, reducing risks of metabolic syndrome, diabetes and cardiovascular disorders (62–66). Moreover, obesity and insulin resistance are associated with pathogenesis of polycystic ovarian syndrome (PCOS), a condition characterized by a reduced frequency of ovulation, infertility, and hyperandrogenism in premenopausal women (67, 68). Metformin’s effect on weight in women with PCOS is not as well defined, depending on the population and study design (69–74). However, a meta-analysis showed that metformin contributed to a decrease of body mass index (BMI) and waist to hip ratio (WHR) in 11 and 7 RCTs of PCOS women respectively, compared to placebo (75). Finally, a 12-week metformin treatment decreased the weight of non-diabetic people living with HIV under antiretroviral therapy (25).
Metformin use has been associated with weight loss and it is noteworthy that metformin-associated weight loss was of a lesser extent in non-diabetic people. As such obese people may benefit from bariatric surgery more readily than from metformin (76). Although most studies have confirmed that metformin could decrease body weight in diabetic and non-diabetic subjects, the mechanism still remains unclear. Aside from the mechanisms summarized in two reviews (77, 78), recent study findings indicate that GDF-15 plays an independent role in body weight change in people taking metformin.

**Metformin Induces GDF-15 Via ATF4 and CHOP**

Converging findings showed that metformin induced gdf-15 gene expression and elevated the circulating GDF-15 level in animal and human models (Table 1). Metformin-induced expression was notably detected in gut and kidney epithelial cells (23). Metformin and other biguanides such as phenformin were shown to induce GDF-15 expression in murine and human hepatocytes (23), while the direct effect of other anti-diabetes drugs has not been studied yet. In vitro, 1 mM of metformin upregulated GDF-15 gene expression in breast cancer cells 26-fold compared with control (79). Higher concentrations of metformin (10-100 mM) in mesenchymal stem cells (MSCs) increased GDF-15 expression in a dose-dependent manner under normoglycemic conditions. Interestingly, this effect was hindered in hyperglycemic conditions (80). In vivo, Gerstein et al. assayed 237 biomarkers in baseline serum from 8,401 participants with dysglycemia of whom 2,317 received metformin and found that GDF-15 was linked to metformin treatment, in a dose dependent manner (1 per mg of metformin treatment led to 8.7 pg/ml of GDF-15 increased in plasma). Moreover, Coll et al. (23) reported in two independent

| Study, year | Models | Number | Dose of metformin | Change of GDF-15 by metformin |
|-------------|--------|--------|------------------|------------------------------|
| **Animal studies** | | | | |
| Day et al., 2019 (24) | Mice fed with chow diet and high fat diet | n = 6-7 per group | A single oral gavage of metformin (250 mg/kg) or an equal volume of saline | Metformin significantly increased serum GDF-15 in both chow diet and high fat diet groups |
| Coll et al., 2020 (23) | Obese mice | Three groups: Vehicle, Metformin (300 mg/kg) | Single oral dose of 300 or 600 mg/kg | 300 mg/kg of metformin increased GDF-15 levels for at least 8 h. 600 mg/kg of metformin resulted in a six-fold increase in serum GDF-15 levels at 4 h and 8 h after the dose. |
| **Cellular studies** | | | | |
| Williams et al., 2013 (79) | MDA-MB-468 breast cancer cells | n = 3 per group | Cells were cultured for 48 h in the absence or presence of 1 mM metformin | GDF-15 gene expression was increased 25.61 fold in metformin group compared with control. |
| Zafarvahedian et al., 2017 (80) | Mesenchymal stem cells (MSCs) | n = 3 per group | MSCs were treated with 10, 50, and 100 mM metformin for 17 h | GDF-15 production was increased in a dose dependent manner. GDF-15 levels increased by dose up to 2-fold control group levels at 100 mM. |
| Day et al., 2019 (24) | Primary mouse hepatocytes | (1) n = 4 per group (2) n = 3-6 per group | (1) Cells were treated with 0.5 mM metformin for 24 h (2) 0-1,000 μM for 24 h | (1) Metformin treatment significantly increased GDF-15 mRNA levels. (2) Metformin increased GDF-15 release in a dose-dependent manner |
| **Clinical trials** | | | | |
| Gerstein et al., 2017 (81) | People with diabetes, impaired glucose tolerance, or impaired fasting glucose levels | 8,401 participants (2,317 receiving metformin) | Various doses | Mean GDF-15 concentrations rose with metformin dose. GDF-15 was strongly linked to metformin, such that the odds of metformin use per standard deviation value increase in level varied from 3.73 (95% CI 3.40, 4.09) to 3.94 (95% CI 3.59, 4.33) depending on included variables. |
| Natali et al., 2018 (82) | Diabetic patients | 644 (Metformin) vs 299 (Non-metformin) | Not mentioned | Metformin treatment was associated with a 40% rise in GDF-15 level, which was independent of the other major factors. |
| Coll et al., 2020-2 (23) | Overweight individuals | 9 (placebo-controlled, double-blind crossover design) | Week 1: 500 mg twice daily; week 2: 1,000 mg twice daily. | After two weeks of metformin treatment, there was an increase of about 2.5-fold in mean circulating GDF-15. |
| Coll et al., 2020-3 (23) | Overweight or obese non-diabetic participants | 88 (Metformin) vs 85 (placebo) | 850 mg daily for 18 months | Metformin treatment was associated with significantly increased levels of circulating GDF-15 at all three time points (6, 12 and 18 months). |
| Isnard et al., 2020 (25) | Non-diabetic People living with HIV | Metformin | 850 mg twice daily for 12 weeks. | Metformin treatment was associated with significantly increased levels of circulating GDF-15 at 12 weeks. Plasma GDF-15 levels went back to baseline levels 12 weeks after metformin discontinuation. |
RCT that metformin reduced food intake and lowered body weight in association with increasing levels of GDF-15. However, the same group showed that metformin retained its ability to lower circulating glucose and fasting insulin levels in GDF-15 knockout mice. These findings suggest that GDF-15 mediates the beneficial effects of metformin on energy balance and weight loss, independently of insulin pathways. However, based on the negative effect of weight on insulin sensitivity, it was speculated that GDF-15-dependent weight loss contributed to enhance insulin sensitivity. We conducted a prospective study to examine the effect of metformin on body weight in non-diabetic, non-obese people living with HIV and receiving effective antiretroviral therapy (ART). We showed that metformin, independent of its glucose-lowering effect, increased plasma levels of GDF-15 and decreased weight, and its effects vanished upon discontinuation, establishing a direct cause-effect relationship between GDF-15 plasma level change and weight change during and after metformin discontinuation (25).

Although the mechanism responsible for metformin-induced GDF-15 expression is not yet deciphered, several pathways have been described to induce GDF-15 expression. In diabetic patients, hyperglycemia causes a stress condition leading to reactive oxygen species (ROS) overproduction, which further induces cellular apoptosis, cellular injury and cell death by inhibiting the PI3K/AKT/eNOS/NO pathway and activating the NF-kB/INK/caspase-3 pathway (83–85). In vitro, Li et al. revealed that high glucose could induce GDF-15 expression and secretion in cultured human umbilical vein endothelial cells in a ROS- and p53-dependent manner (86). The transcriptional factor p53 regulates GDF-15 expression and was shown to link GDF-15 with obesity and insulin resistance (87). Obesity promotes p53 activation in adipose tissue and leads to increased production of proinflammatory cytokines and increased insulin resistance. When p53 was inhibited by RNA silencing, the effect of GDF-15 induction by high glucose vanished (86). In humans, high levels of plasma GDF-15 have been associated with type 2 diabetes and cardiovascular events (88–90). However, GDF-15 was shown to protect human islet cells from apoptosis and was suggested to have a protective role in diabetic mice (91). Metformin is also suggested to prevent cardiovascular diseases and to have anti-aging effects (15, 92–94), although these results need to be confirmed (95). As such, the clinical implication of metformin-induced GDF-15 increase will have to be assessed in future RCTs.

Aside from p53, several other factors have been implicated in the transcriptional regulation of GDF-15, including p63, Sp1, early growth response-1 (EGR-1), activating the integrated stress response transcription factor 4 (ATF4), C/EBP homologous protein (CHOP), and SMAD2/3 (96–103). Patel et al. reported that GDF-15 levels increased following sustained high-fat feeding or dietary amino acid imbalance in mice, and that GDF-15 expression is regulated by the integrated stress response, in which key transcriptional regulators like ATF4 and CHOP are involved (102, 104). Induced GDF-15 expression by the stressor tunicamycin was abolished in ATF4 knockout mouse embryonic fibroblasts and significantly reduced in CHOP-knockdown cells (102). Similarly, Chung et al. (100) showed that induction of GDF-15 upon mitochondrial unfolded protein response (UPRm) activation was CHOP-dependent. Interestingly, metformin was reported to increase the expression of ATF4 and CHOP, further stimulating the secretion of GDF-15 in mouse and human hepatocytes (24). Therefore, current evidence indicates that metformin increases GDF-15 gene expression and stimulate GDF-15 secretion by direct induction of integrated stress response regulators ATF4 and CHOP.

### GDF-15 and Weight Loss: Evidences and Mechanism

A direct association between weight and GDF-15 was studied in animal and humans. Altered GDF-15 levels in comparison to matched lean controls have been frequently reported in obese mice, rats, and humans (32, 105). In addition, elevated GDF-15 expression and circulating levels correlate with further weight loss, reduce food intake and appetite (42, 43, 106). Patients with metastatic lung cancer who reported >5% weight loss were found to exhibit a twofold increase in GDF-15 plasma levels compared to those without weight loss (42). Tsai et al. (107) reported that GDF-15 gene knockout mice (Gdf-15−/−) weighed more and had increased adiposity, which was associated with increased food intake, and that infusion of human recombinant GDF-15 was sufficient to raise serum levels in Gdf-15−/− mice to within the normal human range, reducing body weight and food intake. On the contrary, overexpressing GDF-15 led to decreased body weight, fat mass and food intake, improving the glucose tolerance in mouse models (83, 108–110). GDF-15 has thus become an attractive target for reducing obesity. In line with these studies, exogenous administration of recombinant murine or human GDF-15 induced weight loss in different animal models (26, 32, 40, 41, 107). Xiong et al. (32) showed that Fc fusion GDF-15 molecules with extended half-life and increased efficacy in obese mice, rats, and cynomolgus monkeys was able to delay gastric emptying, change food preference, and activate area postrema neurons, confirming a role for GDF-15 in the gut-brain axis responsible for the regulation of body energy intake. Moreover, pharmacological recombinant human GDF-15 administration to mice can trigger conditioned-taste aversion, suggesting that GDF-15 may induce an aversive response to nutritional stimulation and may be associated with nausea in pregnancy (102). These results were further confirmed by Coll et al. as metformin did not induce weight loss when administered to gdf-15 knockout mice (23).

Mechanistically, the weight-related effect of GDF-15 is dependent on its receptor GFRAL and coreceptor tyrosine kinase RET (Figure 1). Unlike Gdf-15 which is expressed in diverse tissues, including kidney, liver, gut, muscle, adipose, and placenta, GFRAL expression is limited to the brainstem and Gfral mRNA is highly expressed in the area postrema of mouse, rat, monkey and human (39–41). GFRAL was previously considered as an orphan receptor with no endogenous ligand (111, 112). Recently, GDF-15 was validated as the only GFRAL ligand with a high-affinity. Flow cytometry analyses showed that GFRAL solely bound to GDF-15, but not to GDNF and its homologs neurturin, artemin, and persephin (26). Interestingly, GDF-15 also exclusively binds to GFRAL, and not to any other TGF-β receptors nor to other members of the GDNF family of receptors (39–41). In the brain,
activation of the GFRAL receptor leads to a complex activation of a neuronal network involving the nucleus of the solitary tract, the hypothalamus, and the central amygdala, reducing food intake and appetite (Figure 1) (96). Gfral knockout mice are hyperphagic under stressed conditions and are resistant to chemotherapy-induced anorexia and body weight loss. Moreover, the effect of GDF-15 on body weight and food intake reduction in wild-type mice was completely lost in Gfral knockout mice (39). GFRAL antibody blocked GDF-15-induced body weight and food-intake suppression in rats (39). Additionally, GDF-15-induced cell signaling requires the interaction of GFRAL with the coreceptor RET (39). GDF-15 forms a complex with GFRAL and RET on the cell surface, then triggers an intracellular signaling cascade through the extracellular signal-related kinase (ERK) pathway (40, 113). Blocking RET by inhibitor or mRNA depletion could also prevent GDF-15-mediated signaling in neuroblastoma cells (41).

CONCLUSION

Metformin has emerged as an effective weight-reducing medication in different animal and human models by increasing GDF-15 levels, which works as a “weight watcher” to maintain homeostasis. Metformin induces the expression of integrated stress response regulators ATF4 and CHOP, which stimulates the secretion of circulating GDF-15, which then binds to its exclusive receptor GFRAL and coreceptor RET in the brainstem. Intracellular signaling of the GDF-15/GFRAL/RET leads to feelings of satiety and control of appetite, resulting in decreased body weight (Figure 1). However, more evidence is needed to verify the role of GDF-15 as a biomarker of metformin’s weight-reducing effects in diabetic and non-diabetic individuals. In the future, metformin dose and duration should be well-defined, and use GDF-15 as a biomarker of metformin-induced weight loss will have to be confirmed. Moreover, the clinical implication and use of GDF-15 as a biomarker should be studied in large RCTs.

AUTHOR CONTRIBUTIONS

JO and SI wrote the first draft of the manuscript. JL, BF, and XP provided critical revision of the manuscript. YC and J-PR conceived and designed the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

Our research is funded by the Fonds de la Recherche Québec-Santé (FRQ-S): Réseau SIDA/Maladies infectieuses and Thérapie cellulaire, the Canadian Institutes of Health Research (CIHR; grants MOP 103230 and PTJ 166049), the Vaccines &
Immunotherapies Core of the CIHR Canadian HIV Trials Network (CTN; CTN PT027), the Canadian Foundation for AIDS Research (CANFAR; grant 02-512), and CIHR-funded Canadian HIV Cure Enterprise (CanCURE) Team Grant HB2-164064.

REFERENCES

1. Pacioso S, Cresci B, Pala L, Rotella CM, Parenti A. Obesity Therapy: How and Why? Curr Med Chem (2020) 27:174–86. doi: 10.2174/0929867326666190124121725
2. Nijhawans P, Behl T, Bhardwaj S. Angiogenesis in obesity. Biomedi Pharmacother (2020) 126:e110103. doi: 10.1016/j.biopharma.2020.110103
3. American Diabetes Association. Standards of Medical Care in Diabetes-2019 Abridged for Primary Care Providers. Clin Diabetes Publ Am Diabetes Assoc (2019) 37:11–34. doi: 10.2337/cd18-0105
4. Yale JF, Paty B, Senior PA. Hypoglycemia. Can J Diabetes (2018) 42 (Suppl 1):S104–S108. doi: 10.1016/j.cjkd.2017.10.010
5. Harper W, Clement M, Goldenberg R, Hanna A, Retnakaran R, et al. Pharmacologic management of type 2 diabetes. Can J Diabetes (2013) 37(Suppl 1):S61–8. doi: 10.1016/j.jcjd.2013.01.021
6. Bonadonna RC, Yale JF, Brulle-Wohlueter C, Boelle-Le Corfec E, Chertow GR, Pappas TS. Hypoglycemia as a function of HbA1c in type 2 diabetes: Insulin glargine 300 U/mL in a patient-level pooled analysis of EDITION 1, 2, and 3. Diabetes Obes Metab (2019) 21:715–9. doi: 10.1111/dom.13578
7. Pollak M. The effects of metformin on gut microbiota and the immune system as research frontiers. Diabetologia (2017) 60:1662–7. doi: 10.1007/s00125-017-4352-x
8. Kim SA, Choi HC. Metformin inhibits in inflammatory response via AMPK-PTEN pathway in vascular smooth muscle cells. Biochem Biophys Res Commun (2012) 425:866–72. doi: 10.1016/j.bbrc.2012.07.165
9. Hirsch HA, Ilipoulos D, Sreahl K. Metformin inhibits the inflammatory response associated with cellular transformation and cancer stem cell growth. Proc Natl Acad Sci U.S.A. (2013) 110:9727–73. doi: 10.1073/pnas.1220511110
10. Li SN, Wang X, Zeng QT, Feng YB, Cheng X, Mao XB, et al. Metformin modulates human leukocyte/endothelial cell interactions and proinflammatory cytokines in polycystic ovary syndrome patients. Atherosclerosis (2015) 242:167–73. doi: 10.1016/j.atherosclerosis.2015.07.017
11. Victor MV, Rovira-Llopis S, Banuls C, Diaz-Morales N, Lopez-Domenech S, Onken B, Driscoll M. Metformin induces a dietary restriction-like state and the oxidative stress response to extend C. elegans Healthspan via AMPK, LKB1, and fi. derecognition. J. atherosclerosis.2015.07.017
12. Ouyang et al. GDF-15 Mediates Metformin on Weight Change in Type 2 Diabetes: A Systematic Review and Meta-analysis. Ann Intern Med (2016) 164:740–51. doi: 10.7326/M16-2560
13. Diabetes Prevention Program Research Group. Long-term safety, tolerability, and weight loss associated with metformin in the Diabetes Prevention Program Outcomes Study. Diabetes Care (2012) 35:731–7. doi: 10.2337/dc11-1299
14. Ejtabad HS, Tito RY, Siadat SD, Hasani-Ranjbar S, Hoseini-Tavassol Z, Rymenas I, et al. Metformin induces weight loss associated with gut microbiota alteration in non-diabetic obese women: a randomized double-blind clinical trial. Eur J Endocrinol (2018) 180:165–76. doi: 10.1530/EJE-18-0826
15. Rebollo CJ, Greenway FL. Obesity medications in development. Expert Opin Investig Drugs (2020) 29:63–71. doi: 10.1080/13543784.2020.1705277
16. Papathanasiou AE, Nolen-Doerr E, Farr OM, Mantzoros CS. GOFREY HARRIS PRIZE LECTURE 2018: Novel pathways regulating neuroendocrine function, energy homeostasis and metabolism in humans. Eur J Endocrinol (2019) 180:R39–71. doi: 10.1530/EJE-18-0847
17. Coll AP, Chen M, Taskar P, Rimmington D, Patel S, Tadross JA, et al. GDF15 mediates the effects of metformin on body weight and energy balance. Nature (2020) 578:448–48. doi: 10.1038/s41586-019-1911-y
18. Day EA, Ford RJ, Smith BK, Mohammad-Shemirani P, Morrow MR, Gutgesell RM, et al. Metformin-induced increases in GDF15 are important for suppressing appetite and promoting weight loss. Nat Metab (2019) 1:1202–8. doi: 10.1038/s42255-019-0146-4
19. Isnard S, Lin J, Fombuena B, Ouyang J, Varin TV, Richard C, et al. Repurposing metformin in non-diabetic people living with HIV: Influence on weight and gut microbiota. Open Forum Infect Dis (2020) 7. doi: 10.1093/ofid/ofaa338
20. Yang L, Chang CC, Sun Z, Madsen D, Zhu H, Padkjaer SB, et al. GFRAL is the receptor for GDF15 and is required for the anti-obesity effects of the ligand. Nat Med (2017) 23:1158–66. doi: 10.1038/nm.4394
21. Wischhusen J, Melero I, Fridman WH. Growth/Differentiation Factor-15 (GDF-15): From Biomarker to Novel Targetable Immune Checkpoint. Front Immunol (2020) 11:951. doi: 10.3389/fimmu.2020.00959
22. Adela R, Banerjee SK. GDF-15 as a Target and Biomarker for Diabetes and Cardiovascular Disease: A Translational Prospective. J Diabetes Res (2015) 2015:498042. doi: 10.1155/2015/498042
23. Benes J, Kotrc M, Wohlfahrt P, Conrad MJ, Franekova J, Jabor A, et al. The Role of GDF-15 in Heart Failure Patients With Chronic Kidney Disease. Can J Cardiol (2019) 35:462–70. doi: 10.1016/j.cjca.2018.12.027
24. Arkoumani M, Papadopoulou-Marketou N, Nicolaides NC, Kanaka-Gantenbein C, Tentolouris P, Papassotiriou I. The clinical impact of growth differentiation factor-15 in heart disease: A 2019 update. Crit Rev Clin Lab Sci (2020) 57:114–25. doi: 10.1097/CLB.00000000000008765
25. Spanopoulou A, Gkretsi V. Growth differentiation factor 15 (GDF15) in cancer cell metastasis: from the cells to the patients. Clin Exp Metastasis (2020) 37:451–64. doi: 10.1007/s10477-020-10441-3
26. Xiong Y, Walker K, Min X, Hale C, Tran T, Komorowski R, et al. Long-acting MIC-1/GDF15 molecules to treat obesity: Evidence from mice to monkeys. Sci Transl Med (2017) 9:eaan8732. doi: 10.1126/scitranslmed.aan8732
27. You AS, Kalantar-Zadeh K, Lerner I, Nakata T, Lopez N, Lou L, et al. Association of Growth Differentiation Factor 15 with Mortality in a Prospective Hemodialysis Cohort. Am J Cardiorenal Med (2017) 5:1358–68. doi: 10.1159/000455907
28. Husebo G, Gronseth R, Lerner L, Gyriso J, Hardie JA, Bakke PS, et al. Growth differentiation factor-15 is a predictor of important disease outcomes in patients with COPD. Eur Respir J (2017) 49:1601298. doi: 10.1183/13993003.01298-2016
29. Hansen ES, Hindberg K, Latyshev N, Aukrust P, Ueland T, Hansen JB, et al. Plasma levels of growth differentiation factor 15 are associated with future outcomes in patients with COPD. Eur Respir J (2020) 55:1809–18. doi: 10.1183/13993003.01298-2016

ACKNOWLEDGMENTS

We are highly grateful to Angie Massicotte, Josee Giroud, and Cezar Iovi for coordination and assistance.
risk of venous thromboembolism. *Blood* (2020) 136:1865–70. doi: 10.1182/blood.2019004572.

36. Schittenhelm AD, Schober A, Strelau J, Bonattiera GA, Schmidt W, Unsicker K, et al. Involvement of growth differentiation factor-15/macrophage inhibitory cytokine-1 (GDF-15/MIC-1) in exLDL-induced apoptosis of human macrophages in vitro and in arteriosclerotic lesions. *Cell Tissue Res* (2004) 318:325–33. doi: 10.1007/s00441-004-0986-3.

37. Kempf T, Zarbock A, Widera C, Butz S, Stadtmann A, Rossaint J, et al. GDF-15 is an inhibitor of leukocyte integrin activation required for survival after myocardial infarction in mice. *Nat Med* (2011) 17:581–8. doi: 10.1038/nm.2354.

38. Kempp T, Eden M, Strelau J, Naguib M, Willenbocle C, Tongers J, et al. The transforming growth factor-beta superfamily member growth-differentiation factor-15 protects the heart from ischemia/reperfusion injury. *Circ Res* (2006) 98:351–60. doi: 10.1161/01.RES.0000202805.73038.48.

39. Emmerson PJ, Wang F, Du Y, Liu Q, Pickard RT, Gonciarz MD, et al. The metabolic effects of GDF15 are mediated by the orphan receptor GFRAL. *Nat Med* (2017) 23:1215–9. doi: 10.1038/nm.4393.

40. Hsu JY, Crawley S, Chen M, Ayupova DA, Lindhout DA, Higbee J, et al. Non-homeostatic body weight regulation through a brainstem-restricted GDF-15 expressed in mice. *J Psychopharmacol (Oxford England)* (2016) 30:1185–96. doi: 10.1177/0269881116645420.

41. Millucan SE, Lin-Schmidt X, Chin CN, Chavez JA, Furman JL, Armstrong AA, et al. GFRAL is the receptor for GDF15 and the ligand promotes weight loss in mice and nonhuman primates. *Nat Med* (2017) 23:1150–7. doi: 10.1038/nm.4392.

42. Lerner L, Gyuris J, Nicoletti R, Gifford J, Krieger B, Jatoi A. Growth differentiating factor-15 (GDF-15): A potential biomarker and therapeutic target for cancer-associated weight loss. *OncoLett* (2016) 12:4219–23. doi: 10.3989/ol.2016.1518.

43. Lerner L, Hayes TG, Tao N, Krieger B, Feng B, Wu Z, et al. Plasma growth differentiation factor 15 is associated with weight loss and mortality in cancer patients. *J cachexia sarcopenia Muscle Metabol* (2020) 11:175–82. doi: 10.1002/jjem.2020005208.

44. Golay A, Ybarra J. Link between obesity and type 2 diabetes. Best Practice & Research: Clinical Endocrinology & Metabolism (2005) 19:591–607. doi: 10.1016/j.cem.2005.07.010.

45. The Diabetes Prevention Program Research Group. The Diabetes Prevention Program: baseline characteristics of the randomized cohort. The Diabetes Prevention Program Research Group. *Diabetes Care* (2000) 23:1619–29. doi: 10.2327/diacare.23.11.1619.

46. Daousi C, Casson IF, Gill GV, MacFarlane IA, Wilding JP, Pinkney JH. Prevalence of obesity in type 2 diabetes in secondary care: association with cardiovascular risk factors. *Postgrad Med J* (2006) 82:280–4. doi: 10.1136/pmj.2005.039302.

47. Zumbra A, Niederman MS. The explosive epidemic outbreak of novel coronavirus disease 2019 (COVID-19) and the persistent threat of transmitted respiroinfectious diseases to global health security. *Curr Opin Pulm Med* (2020) 26:2193–6. doi: 10.1097/MCP.0000000000001676.

48. Fonseca V. Effect of thiazolidinediones on body weight in patients with diabetes mellitus. *Am J Med* (2003) 115(Suppl 8A):42s–8s. doi: 10.1016/j.amjmed.2003.09.005.

49. Kahn SE, Haffner SM, Heise MA, Herman WH, Holman RR, Jones NP, et al. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *N Engl J Med* (2005) 353:2427–34. doi: 10.1056/NEJMoa0466224.

50. Saenz A, Fernandez-Esteban I, Mataix A, Ausejo M, Roque M, Roque H, et al. Metformin monotherapy for type 2 diabetes mellitus. *Cell Tissue Resys* (2005) 56:1301–2. doi: 10.1001/jcm.12033.

51. Zumbra A, Niederman MS. The explosive epidemic outbreak of novel coronavirus disease 2019 (COVID-19) and the persistent threat of transmitted respiroinfectious diseases to global health security. *Curr Opin Pulm Med* (2020) 26:2193–6. doi: 10.1097/MCP.0000000000001676.

52. Fonseca V. Effect of thiazolidinediones on body weight in patients with diabetes mellitus. *Am J Med* (2003) 115(Suppl 8A):42s–8s. doi: 10.1016/j.amjmed.2003.09.005.

53. Kahn SE, Haffner SM, Heise MA, Herman WH, Holman RR, Jones NP, et al. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *N Engl J Med* (2006) 353:2427–34. doi: 10.1056/NEJMoa0466224.

54. Saenz A, Fernandez-Esteban I, Mataix A, Ausejo M, Roque M, Roque H, et al. Metformin monotherapy for type 2 diabetes mellitus. *Cell Tissue Resys* (2005) 56:1301–2. doi: 10.1001/jcm.12033.

55. Garvey WT, Mechanick JI, Brett EM, Garber AJ, Hurley DL, Jastreboff AM, et al. American Association Of Clinical Endocrinologists And American College Of Endocrinology Comprehensive Clinical Practice Guidelines For Medical Care Of Patients With Obesity. *Endocr Pract Off J Am Coll Endocrinol Am Assoc Clin Endocrinologists* (2016) 22(Suppl 3):1–203. doi: 10.14188/EP161365.GL.
72. Jensterle Sever M, Kocjan T, Pfeifer M, Kravos NA, Janez A. Short-term combined treatment with iraglutide and metformin leads to significant weight loss in obese women with polycystic ovary syndrome and previous poor response to metformin. *Eur J Endocrinol* (2014) 170:451–9. doi: 10.1530/EJE-13-0797

73. Al-Nozha O, Habib F, Mojaddidi M, El-Bah MF. Body weight reduction and metformin: Roles in polycystic ovary syndrome. *Pathophysiology* (2013) 20:131–7. doi: 10.1016/j.pathophys.2013.03.002

74. Aghahosseini M, Ayleasem A, Saffaridan I, Moddarees-Hashemi S, Mofid B, Kashani L. Metformin 2.500 mg/day in the treatment of obese women with polycystic ovary syndrome and its effect on weight, hormones, and lipid profile. *Arch Gynecol Obstet* (2010) 282:691–4. doi: 10.1007/s00404-010-1579-x

75. Patel R, Shah G. Effect of metformin on clinical, metabolic and endocrine outcomes in women with polycystic ovary syndrome: a meta-analysis of randomized controlled trials. *Curr Med Res Opin* (2017) 33:1545–57. doi: 10.1080/03007995.2017.1279597

76. Cheng J, Gao J, Shuai X, Wang G, Tao K. The comprehensive summary of surgical versus non-surgical treatment for obesity: a systematic review and meta-analysis of randomized controlled trials. *Oncotarget* (2016) 7:39216–30. doi: 10.18632/oncotarget.9581

77. Malin SK, Kashyap SR. Effects of metformin on weight loss: potential mechanisms. *Curr Opin Endocrinol Diabetes Obes* (2014) 21:323–9. doi: 10.1097/MED.0000000000000095

78. Veravanimal A, Soodak AA. Metformin: Mechanisms in Human Obesity and Weight Loss. *Curr Opes Res* (2019) 8:156–64. doi: 10.1017/S1369-0199-00335-3

79. Williams CC, Singleton BA, Loope SD, Skripnikova EV. Metformin induces a senescence-associated gene signature in breast cancer cells. *J Health Care Poor UnderServed* (2013) 24:93–103. doi: 10.1353/hpu.2013.0014

80. Zafarvahedian E, Roohi A, Sepand MR, Ostad SN, Ghahremani MH. Effect of metformin and celecoxib on cytotoxicity and release of GDF-15 from poor under-served human mesenchymal stem cells in high glucose condition. *Apoptosis* (2017) 21:280–10. doi: 10.1002/cbf.201814200

81. Afanas’ev I, Roohi A, Sepand MR, Ostad SN, Ghahremani MH. Effect of metformin and celecoxib on cytotoxicity and release of GDF-15 from poor under-served human mesenchymal stem cells in high glucose condition. *Apoptosis* (2017) 21:280–10. doi: 10.1002/cbf.201814200

82. Ouyang et al. GDF-15 Mediates Metformin on Weight Loss in Obese Women with Polycystic Ovary Syndrome. *Diabetes Care* (2020) 43:1333–40. doi: 10.1038/sj/diab.2020.04.001

83. Ouyang et al. GDF-15 Mediates Metformin on Weight Loss in Obese Women with Polycystic Ovary Syndrome. *Diabetes Care* (2020) 43:1333–40. doi: 10.1038/sj/diab.2020.04.001

84. Panahi et al. Growth Differentiation Factor 15 as a Novel Biomarker for Metformin. *Cell Rep* (2014) 24:1522–30. doi: 10.1016/j.celrep.2014.02.001

85. Ouyang J, Isnard S, Lin J, Fombuena B, Marette A, Routy B, et al. Metformin effect on gut microbiota: insights for HIV-related inflammation. *AIDS Res Ther* (2017) 14:170. doi: 10.1186/s12981-017-00267-2

86. Han Y, Xie H, Liu Y, Gao P, Yang X, Shen Z. Effect of metformin on all-cause and cardiovascular mortality in patients with coronary artery diseases: a systematic review and an updated meta-analysis. *Cardiovas Diabetol* (2019) 18:96. doi: 10.1186/s12933-019-0433-7

87. Kulkarni AS, Gubbi S, Barzilai N. Benefits of Metformin in Attenuating the Hallmarks of Aging. *Cell Metab* (2020) 32:15–30. doi: 10.1016/j.cmet.2020.04.001

88. Griffin SJ, Leaver JK, Irving GI. Impact of metformin on cardiovascular disease: a meta-analysis of randomised trials among people with type 2 diabetes. *Diabetologia* (2017) 60:1620–9. doi: 10.1007/s00125-017-4337-9

89. He X, Su J, Ma X, Lu W, Zhu W, Wang Y, et al. The association between serum GDF-15 and weight loss in obese women with polycystic ovary syndrome and previous poor response to metformin. *Eur J Endocrinol* (2014) 170:451–9. doi: 10.1530/EJE-13-0797

90. Chung HK, Ryu D, Kim KS, Chang JY, Kim YK, Yi HS, et al. Growth differentiation factor 15 is a myostatine governing systemic energy homeostasis. *J Cell Biol* (2017) 216:1469–65. doi: 10.1083/jcb.201607110

91. Jones JE, Cadena SM, Gong C, Wang X, Chen Z, Wang SX, et al. Supraphysiologic Administration of GDF11 Induces Cachexia in Part by Uregulating GDF15. *Cell Rep* (2018) 22:1522–30. doi: 10.1016/j.celrep.2018.01.044

92. Patel S, Alvarez-Guita A, Melvin A, Rimmington D, Battilo A, Miedzybrodzka EL, et al. GDF11 Provides an Endocrine Signal of Nutritional Stress in Mice and Humans. *Cell Metab* (2019) 29:707–718.e8. doi: 10.1016/j.cmet.2018.12.016

93. Ichikawa T, Suenaga Y, Koda T, Ozaki T, Nakagawara A. TAp63-dependent inflammatory drug-activated gene promoter. Basal transcription is mediated by Sp1 and Sp3. *J Biol Chem* (2001) 276:33384–92. doi: 10.1074/jbc.M01814200

94. Osada M, Park HL, Park MJ, Liu JW, Wu G, Trink B, et al. A p53-type response element in the GDF15 promoter confers high specificity for p53 activation. *Biochem Biophys Res Comm* (2007) 354:913–8. doi: 10.1016/j.jbrc.2007.01.089

95. Lim JH, Park JW, Min DS, Chang JS, Lee YH, Park YB, et al. NAG-1 up-regulation mediated by EGR-1 and p53 is critical for quercetin-induced apoptosis in HCT116 colon carcinoma cells. *Apoptosis an Int J Programmed Cell Death* (2007) 12:411–21. doi: 10.1007/s00125-006-0576-9

96. Bao X, Borne Y, Muhammad IF, Lin J, Bobadilla H, et al. Tumor-inhibitory effect of metformin and celecoxib on cytotoxicity and release of GDF-15 from poor undervilled human mesenchymal stem cells in high glucose condition. *Apoptosis* (2017) 22:1522–30. doi: 10.1016/j.celrep.2018.01.044

97. Kempf T, Guba-Quint A, Torgerson J, Magnone MC, Hae ger C, Bobadilla H, et al. Tumor-inhibitory effect of metformin and celecoxib on cytotoxicity and release of GDF-15 from poor undervilled human mesenchymal stem cells in high glucose condition. *Apoptosis* (2017) 22:1522–30. doi: 10.1016/j.celrep.2018.01.044

98. Ouyang et al. GDF-15 Mediates Metformin on Weight Loss in Obese Women with Polycystic Ovary Syndrome. *Diabetes Care* (2020) 43:1333–40. doi: 10.1038/sj/diab.2020.04.001

99. Chen J, Hou G, Hou W, et al. The association between serum GDF-15 and weight loss in obese women with polycystic ovary syndrome and previous poor response to metformin. *Eur J Endocrinol* (2014) 170:451–9. doi: 10.1530/EJE-13-0797
109. Baek SJ, Okazaki R, Lee SH, Martinez J, Kim JS, Yamaguchi K, et al. Nonsteroidal anti-inflammatory drugactivated gene-1 overexpression in transgenic mice suppresses intestinal neoplasia. *Gastroenterology* (2006) 131:15360. doi: 10.1053/j.gastro.2006.09.015

110. Macia L, Tsai VW, Nguyen AD, Johnen H, Kuffner T, Shi YC, 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* (2012) 7:e34868. doi: 10.1371/journal.pone.0034868

111. Häätinen T, Holm L, Airaksinen MS. Loss of neurturin in frogcomparative genomics study of GDNF family ligand-receptor pairs. *Mol Cell Neurosci* (2007) 34:15567. doi: 10.1016/j.mcn.2006.10.009

112. Airaksinen MS, Holm L, Häätinen T. Evolution of the GDNF family ligands and receptors. *Brain Behav Evol* (2006) 68:18190. doi: 10.1159/000094087

113. Asai N, Murakami H, Iwashita T, Takahashi M. A mutation at tyrosine 1062 in MEN2A-Ret and MEN2B-Ret impairs their transforming activity and association with shc adaptor proteins. *J Biol Chem* (1996) 271:176449. doi: 10.1074/jbc.271.30.17644

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Ouyang, Isnard, Lin, Fombuena, Peng, Chen and Routy. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.