Metabolic hormones and ethnic aspects in obesity
Hoekx, C.A.

CHAPTER 7

GROWTH DIFFERENTIATION FACTOR 15 IS NOT MODIFIED AFTER WEIGHT LOSS INDUCED BY LIRAGLUTIDE IN SOUTH ASIANS AND EUROPIDS WITH TYPE 2 DIABETES MELLITUS

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ABSTRACT

Objectives
Glucagon-like peptide-1 receptor (GLP-1R) agonists induce weight loss in patients with type 2 diabetes mellitus (T2DM), but the underlying mechanism is unclear. Recently, the mechanism by which metformin induces weight loss could be explained by an increase in the growth differentiation factor 15 (GDF15), which suppresses appetite. Therefore, we aimed to investigate whether the GLP-1R agonist liraglutide modifies plasma GDF15 levels in patients with T2DM.

Methods
GDF15 levels were measured in plasma samples obtained from Dutch Europids and Dutch South Asians with T2DM before and after 26 weeks of treatment with daily liraglutide (n=44) or placebo (n=50) added to standard care.

Results
At baseline, circulating GDF15 levels did not differ between South Asians and Europids with T2DM. Treatment with liraglutide, compared to placebo, decreased body weight, but did not modify plasma GDF15 levels in all patients, or when data were split by ethnicity. Also, the change in plasma GDF15 levels after treatment with liraglutide did not correlate with changes in body weight or HbA_{1c} levels. In addition, the dose of metformin used did not correlate with baseline plasma GDF15 levels.

Conclusion
Compared to placebo, liraglutide treatment for 26 weeks does not modify plasma GDF15 levels in Dutch Europid or South Asian patients with T2DM. Thus, the weight loss induced by liraglutide is likely explained by other mechanisms beyond the GDF15 pathway.

INTRODUCTION

The number of people living with type 2 diabetes mellitus (T2DM) has rapidly increased globally in recent years and is expected to continue to rise (1). Obesity is a major risk factor for the development of T2DM (2). South Asian ethnicity is another well-known risk factor, as South Asians have a significantly higher risk of developing T2DM at a younger age and a lower body mass index (BMI) than other ethnic groups, including Europids (3). The underlying mechanism for this increased risk of developing T2DM in South Asians is not entirely known; however, it is likely multifactorial. Their disadvantageous body composition, consisting of a higher fat mass percentage with more visceral adipose tissue and a lower muscle mass than Europids, is a significant contributing factor to a higher insulin resistance state (4-6). Hormonal cues may also play a role in the increased risk of developing T2DM in South Asians. Differences in appetite-regulating hormones such as glucagon-like peptide-1, leptin, and ghrelin have previously been described in South Asians compared to other ethnicities (7-9). These hormonal variations could further contribute to the increased risk of South Asians to develop T2DM.

Metformin is the first-line pharmacological treatment for patients with new-onset T2DM. It acts by reducing hepatic gluconeogenesis, enhancing peripheral insulin sensitivity, and increasing the secretion of the gut hormone GLP-1, ultimately leading to reduced plasma glucose levels. Besides improving glucose regulation, metformin also reduces body weight (10, 11). Recent translational studies from two independent research groups have demonstrated that metformin increases circulating growth differentiation factor 15 (GDF15) levels to suppress appetite, thereby inducing weight loss (12, 13). GDF15 is a stress-induced cytokine and a member of the transforming growth factor-beta superfamily (14). It induces satiety by binding to the glial cell line-derived neurotrophic factor (GDNF) family Receptor alpha-like (GFRAL) located in the area postrema and solitary tract of the hindbrain. GFRAL subsequently interacts with the tyrosine kinase co-receptor RET which induces phosphorylation of the signaling molecules AKT, ERK1/2, and phospholipase C, thereby inducing anorexia (15-20). In addition, GDF15 induces satiety by signalling through satiety-inducing cholecystokinin neurons located in the hindbrain (19, 21). Clinical trials involving individuals with overweight and obesity who were treated with a long-acting GDF15 receptor analogue showed a reduction in food intake and body weight, supporting the weight-reducing effect of GDF15 (22). Beyond appetite suppression, GDF15 also contributes to weight loss by enhancing energy expenditure, at least in mice (19, 23). Accordingly, metformin also increases energy expenditure in preclinical studies, which was mechanistically at least in part through enhancing activation of energy-combusting brown adipose tissue (24, 25).

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Despite its effectiveness, metformin monotherapy is insufficient in some patients with T2DM to maintain glucose regulation (26). Therefore, additional therapeutic strategies have been developed to improve glycaemic parameters, reducing the risk of T2DM-associated complications. One of these therapies is GLP-1 receptor (GLP-1R) agonism, which mimics the effects of the incretin hormone GLP-1 to stimulate glucose-dependent insulin secretion and reduce glucagon secretion, both contributing to its glucose-lowering effects (27, 28). In addition, GLP-1R agonism has been shown to induce weight loss in patients with T2DM to a greater extent than metformin (29, 30), and its weight loss effect is partly attributed to its ability to promote satiety (31).

Unlike metformin, the underlying mechanism of appetite suppression due to GLP-1R agonism is not entirely known. GLP-1 receptors are expressed throughout the hindbrain, including the area postrema and nucleus solitary tract where GLP-1 receptor agonism induces appetite suppression. Of note, these regions also harbor GFRAL receptors, which mediate the effects of GDF15 in inducing satiety and reducing food intake, as mentioned above (32, 33). This spatial overlap in receptor expression raises the question of whether GLP-1R agonism may modulate the GFRAL/GDF15 signaling pathways and thereby contribute to its appetite-suppressing and weight-reducing effects.

Understanding the mechanism involved in appetite suppression following GLP-1R agonism is of great significance, considering its increasing popularity as a treatment for both diabetes and obesity. Furthermore, with approximately 20% of the world's population of South Asian descent and considering their markedly increased risk to develop obesity and type 2 diabetes compared to subjects of Europid descent, studying potential differences in the GFRAL/GDF15 system in South Asians compared to Europids as a potential underlying mechanism is relevant as well.

Therefore, in the current study, we aimed to study 1) whether circulating GDF15 levels differ between Dutch South Asian and Dutch Europid patients with T2DM; 2) whether the GLP-1R agonist liraglutide modifies plasma levels of GDF15 in both ethnicities and 3) whether changes in GDF15 levels are related to the reduction in body weight after liraglutide treatment in both ethnicities.

METHODS

Participants and study design

Participants
This study is a secondary analysis of two previously performed double-blind, placebo-controlled, randomized clinical trials that were both designed to study the effect of treatment with liraglutide for 26 weeks on glucose regulation and cardiovascular endpoints in patients with overweight and obesity and T2DM (34, 35) and performed at the Leiden University Medical Center (LUMC). In total, 50 patients of Dutch Europid (hereinafter: 'Europid') origin (study 1) (34) and 47 participants of Dutch South Asian (hereinafter: 'South Asian') origin (study 2) (35) were included. South Asian ethnicity is defined as having four grandparents who originally descended from either Surinam, Bangladesh, India, Nepal, Pakistan, Afghanistan, Bhutan, or Sri Lanka. Inclusion criteria were males and females aged 18-69 years, BMI ≥ 25 kg/m^2, and HbA$_{1c}$ levels of 7.0-10.0% (53-86 mmol/mol) despite the use of metformin and/or sulfonylurea derivatives and/or insulin. General exclusion criteria were the use of other glucose-lowering medication than mentioned above, a history of renal or hepatic disease, surgery, pancreatitis, pregnancy or lactation, and the presence of any contra-indication for magnetic resonance imaging (MRI). Both trials were performed between 2013 and 2018.

Study approval
Both studies were performed by the principles of the Declaration of Helsinki (36) and approved by the local ethics committee of the Leiden University Medical Center, Leiden, the Netherlands. All participants provided written informed consent before participation. The trials were registered at ClinicalTrials.gov (registration no. NCT01761318 and NCT02660047).

Treatment regimen
After inclusion, all participants were randomized to receive daily treatment with liraglutide (Victoza®, Novo Nordisk A/S, Bagsværd, Denmark) or placebo (provided by Novo Nordisk A/S, Bagsværd, Denmark) with 1:1 stratification for sex and insulin use. At baseline, the dose of liraglutide was 0.6 mg per day (administered subcutaneously), which was titrated in two steps in three weeks to the maximum amount of 1.8 mg once daily. The dose was reduced, if necessary, in case of adverse events. During the study, participants were contacted weekly by telephone to assess adverse events and to discuss glucose management. During the entire 26 weeks, regular treatment options for optimal glycemic control, and regulation of cholesterol levels and blood pressure were given according to current clinical guidelines. Two Europid participants discontinued

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treatment. One of these participants was in the liraglutide group and discontinued treatment due to repeated hypoglycemic events and was later diagnosed with type 1 diabetes mellitus. The other participant was in the placebo group and could not continue treatment because he was in detention. There were no serious adverse events related to study drug use, and adverse events reported were mild gastrointestinal problems (i.e., nausea and vomiting).

Study design

Extensive descriptions of the design of both trials have been published elsewhere (34, 35). In short, at baseline and after 26 weeks of treatment, all included participants arrived at the outpatient clinic after at least a 6-hour fast. First, body weight, body composition, and lean body mass were assessed by bioelectrical impedance analysis (BIA; scale Bodystat 1500, Bodystat Ltd., Douglas, UK). Then, venous blood samples were collected, and the participants underwent an MRI and proton magnetic resonance spectroscopy (^{1}H-MRS) to measure subcutaneous, visceral, epicardial, and paracardial adipose tissue volume.

Blood collection

After the collection of venous blood samples, serum and plasma were obtained by centrifugation and stored in the freezer at -80°C until analysis. Serum levels of total cholesterol, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured on a Modular P800 analyzer (Roche Diagnostic, Mannheim, Germany). Due to logistical reasons, HbA_{1c} was initially measured with boronate-affinity high-performance liquid chromatography (Primus Ultra; Siemens Healthcare Diagnostics, Breda, the Netherlands) and later with ion-exchange high-performance liquid chromatography (Tosoh G8, Sysmex Nederland B.V., Etten-Leur, the Netherlands). To ensure accurate and consistent results, HbA_{1c} levels obtained from the boronate affinity method were corrected based on the correlation coefficient obtained from validation samples measured on both analyzers. Plasma GDF15 levels were measured with Human Magnetic bead-based multiplex for the Luminex platform (LXSAHM; R&D systems, Minneapolis, Minnesota, USA) according to the manufacturer's protocol.

MRI

At baseline and after 26 weeks of treatment with liraglutide or placebo, participants underwent an MRI in the supine position, using a 3.0 Tesla MRI scanner (Ingenia, Philips Healthcare, Best, the Netherlands) to assess epicardial and paracardial adipose tissue as well as visceral and abdominal adipose tissue volumes, as extensively described previously (35).

Statistical Analyses

Data are expressed as means ± standard deviation. Data normality was confirmed using the Shapiro-Wilk test, visual histograms, and Q-Q plots. Baseline characteristics were compared between the treatment groups and ethnicities using the Chi-square test for binary values (i.e., sex and use of diabetes medication), independent t-test, and Mann-Whitney U for normality distributed data. Not normally distributed data were log10 transformed (e.g., baseline creatine, baseline subcutaneous adipose tissue, visceral adipose tissue, subcutaneous/visceral adipose tissue ratio, paracardial adipose tissue, total cholesterol, and LDL-C). Non-parametric tests were performed on data that were not normally distributed after log10 transformation (e.g., diabetes duration, baseline fat percentage, baseline $HbA_{1c,}$ and metformin dose at baseline). To study the difference between circulating GDF15 levels between ethnicities, an independent t-test was performed with log10 transformed data of baseline GDF15 levels to follow a normal distribution. A delta (Δ; 26-week treatment *minus* baseline value) was created for every outcome. To study the effect of liraglutide on plasma GDF15 levels, body weight, and $HbA_{1c,}$ an analysis of covariance (ANCOVA) was performed, adjusting for baseline values. Moreover, to examine the association of baseline and Δ plasma GDF15 levels with Δ body weight, Δ HbA_{1c}, metformin dose, Δ glucose, Δ total cholesterol, Δ adipose tissue deposition, and Δ kidney function, nonparametric Spearman-rank correlations (rho) were applied. All statistical analyses were performed using the Statistical Package for the Social Sciences v.25.0 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.), whereas all graphs were created with GraphPad Prism software version 9.3.1 for Windows (GraphPad Software, San Diego, California, USA). Significance was set at P < 0.05.

RESULTS

Baseline characteristics

At baseline, no significant differences in age, BMI, sex distribution, or other cardiometabolic parameters were observed between participants receiving liraglutide or placebo in both Europid and South Asian individuals with T2DM, as described previously for both trials (34, 35) (**Table 1**). However, waist circumference (P = 0.029), paracardial (P = 0.013), and pericardial (P = 0.039) adipose tissue volumes were higher in participants from the liraglutide group than those from the placebo group at baseline when both ethnicities were combined (35). When comparing the baseline characteristics between Europid and South Asian individuals, age (P = 0.013), body weight (P < 0.001), body length (P < 0.001), BMI (P = 0.001), waist circumference (P < 0.001), waist to hip ratio (P = 0.001), visceral (P = 0.007), paracardial (P < 0.001), and pericardial adipose tissue volumes (P < 0.001), total cholesterol (P = 0.003), LDL-C (P = 0.022) and metformin dose (P = 0.031) were significantly higher in Europids compared to South Asians. Conversely, South Asians had a longer duration of T2DM compared to the Europids (**Table 1**).

Table 1. Baseline characteristics

	Europids		South Asians		Combined	
	Placebo (n=25)	**Liraglutide (n=22)**	**Placebo (n=25)**	**Liraglutide (n=22)**	**Placebo (n=50)**	**Liraglutide (n=44)**
Demographics						
Females, n, %	n=11, 44%	n=9, 41%	n=14, 56%	n=14, 64%	n=25, 50%	n=23, 52%
Age, years	58.9 ± 6.7	59.9 ± 6.4	54.6 ± 9.4	55.2 ± 11.1	56.7 ± 8.4	57.5 ± 9.2
Diabetes duration, years	10.5 ± 6.9	10.4 ± 5.0	17.0 ± 9.8##	18.8 ± 10.3##	13.8 ± 9.0	14.6 ± 9.0
Clinical parameters						
Body weight, kg	93.8 ± 12.9	98.6 ± 14.1	77.8 ± 12.4###	81.9 ± 11.0####	85.8 ± 14.9	90.3 ± 15.1
Body length, cm	172.4 ± 9.7	173.4 ± 7.8	164.8 ± 9.4##	164.3 ± 8.1###	168.6 ± 10.2	168.9 ± 9.1
BMI, kg/m2	31.5 ± 3.5	32.8 ± 4.3	28.6 ± 4.0##	30.4 ± 3.8	30.1 ± 4.0	31.6 ± 4.2
Waist circumference, cm	108.4 ± 8.1	111.9 ± 9.6	98.4 ± 10.1###	104.1 ± 7.8* ##	103.4 ± 10.4	108.0 ± 9.5*
Hip circumference, cm	106.2 ± 6.7	108.5 ± 8.5	104.0 ± 9.0	104.3 ± 7.1	105.1 ± 7.9	106.4 ± 8.0
Waist to hip ratio	1.0 ± 0.1	1.0 ± 0.1	0.9 ± 0.1##	1.0 ± 0.1*	1.0 ± 0.1	1.0 ± 0.1
Body fat percentage, %	36.7 ± 9.0	36.7 ± 10.1	36.9 ± 9.8	37.2 ± 8.4	36.8 ± 9.3	37.0 ± 9.2
Subcutaneous adipose tissue, cm2	330 ± 109	367 ± 143	326 ± 141	316 ± 97	328 ± 125	341 ± 123
Visceral adipose tissue, cm2	200 ± 62	211 ± 88	149 ± 49##	187 ± 57*	174 ± 61	199 ± 74
Visceral/subcutaneous adipose tissue ratio	0.7 ± 0.3	0.7 ± 0.4	0.5 ± 0.3	0.7 ± 0.3	0.6 ± 0.3	0.7 ± 0.3
Epicardial adipose tissue, cm2	9.6 ± 4.1	8.9 ± 4.4	9.1 ± 2.7	10.4 ± 3.2	9.3 ± 3.4	9.6 ± 3.9
Paracardial adipose tissue, cm2	20.6 ± 10.0	25.8 ± 11.2	9.0 ± 4.5###	12.3 ± 4.4*###	14.4 ± 9.5	19.1 ± 10.8*
Pericardial adipose tissue, cm2	30.2 ± 12.3	34.7 ± 13.7	18.2 ± 5.6###	22.7 ± 6.5* ##	23.9 ± 11.1	28.7 ± 12.2*
HbA1c, mmol/mol	64.6 ± 10.3	66.5 ± 11.7	70.5 ± 12.1	64.8 ± 9.7	67.5 ± 11.5	65.7 ± 10.7
Total cholesterol, mmol/L	4.8 ± 1.0	4.9 ± 1.0	4.5 ± 1.1	4.0 ± 0.7##	4.6 ± 1.1	4.4 ± 1.0
HDL-C, mmol/L	1.3 ± 0.4	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	1.3 ± 0.3	1.2 ± 0.3
LDL-C, mmol/L	2.5 ± 0.9	2.6 ± 0.9	2.2 ± 1.0	2.0 ± 0.7##	2.4 ± 1.0	2.4 ± 0.9

Table 1. Baseline characteristics (continued)

	Europids		South Asians		Combined	
	Placebo (*n*=25)	**Liraglutide (*n*=22)**	**Placebo (*n*=25)**	**Liraglutide (*n*=22)**	**Placebo (*n*=50)**	**Liraglutide (*n*=44)**
Diabetes medication						
Metformin use, n, %	*n*=25, 100%	*n*=22, 100%	*n*=23, 92%	*n*=22, 100%	*n*=48, 96%	*n*=44, 100 %
Metformin, mg/day	1982 ± 553	2093 ± 700	1728 ± 643	1750 ± 665	1860 ± 605	1922 ± 697
Sulfonylurea, n, %	*n*=8, 32%	*n*=6, 27%	*n*=5, 20%	*n*=3, 14%	*n*=13, 26%	*n*=9, 21%
Insulin use, n, %	*n*=16, 64%	*n*=14, 64%	*n*=19, 76%	*n*=17, 77%	*n*=35, 70%	*n*=31, 71%

Adapted from the original data from treatment in Europids (34), and South Asians (35). Two Europids were not included in the analyses since they discontinued treatment. BMI, body mass index; HbA$_{1c}$, hemoglobin A1c; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; Asterisk signs (*) indicate significant differences between treatments within a specific ethnicity, and hash signs (#) indicate significant differences between ethnicities within a specific treatment group. *P < 0.05, #P < 0.05, ##P < 0.01, ###P < 0.001. Data are presented as mean ± standard deviation.

Liraglutide reduces body weight but not HbA$_{1c}$ levels

As previously published, 26 weeks of liraglutide treatment, as compared to placebo, decreased body weight in both Europids (-4.3 ± 3.8 kg *vs.* +0.1 ± 2.5 kg*;* P < 0.001) (34) and South Asians (-3.9 ± 3.6 kg *vs.* -0.6 ± 2.2 kg; P < 0.001) (35). The reduction in body weight induced by liraglutide was not different between ethnicities (P = 0.287). In addition, as previously published, 26 weeks of liraglutide treatment, as compared to placebo, did not decrease HbA$_{1c}$ in both Europids (-11.6 ± 11.1 mmol/mol *vs.* -7.7 ± 9.4 mmol/mol; P = 0.265) (34) and South Asians (-8.5 ± 11.2 mmol/mol *vs.* -6.8 ± 9.3 mmol/mol; P = 0.156) (35).

**Liraglutide does not modify plasma GDF15 levels**

Next, we assessed plasma GDF15 levels at baseline and after 26 weeks of liraglutide treatment and ethnic differences herein. At baseline, plasma GDF15 levels were similar between the liraglutide and placebo groups in both Europids (1495 ± 838 pg/mL *vs.* 1702 ± 1056 pg/mL; P = 0.651) and South Asians (1834 ± 790 pg/mL *vs.* 1814 ± 825 pg/mL; P = 0.774). We combined the baseline GDF15 levels from the participants assigned to the liraglutide and placebo groups to increase the sample size. We observed that baseline levels of GDF15 were not statistically different in South Asians compared to Europids (1823 ± 800 pg/mL *vs.* 1605 ± 956 pg/mL; P = 0.077, **Fig. 1**). Additionally, we performed sensitivity analyses by splitting the data by sex but did not observe significant differences (data not shown).

Twenty-six weeks of treatment with liraglutide, as compared to placebo, did not affect the change (i.e., 26 weeks *minus* baseline) in plasma GDF15 levels in either Europids (-124 ± 962 pg/mL *vs.* -55 ± 911 pg/mL; P = 0.403, **Fig. 2A**), South Asians (-163 ± 853 pg/mL *vs.* -74 ± 732 pg/mL; P = 0.695, **Fig. 2B**). To increase the statistical power, we combined the data from both ethnicities. However, we still did not find significant differences in plasma GDF15 levels between the placebo and liraglutide groups (-144 ± 899 pg/mL *vs.* -64 ± 818 pg/mL *vs.* liraglutide; P = 0.386, **Fig. 2C**). Additionally, we repeated all analyses separately for men and women, and for men and women with different ethnicities and the lack of effect also persisted (**Figs. 3A** and **B**).
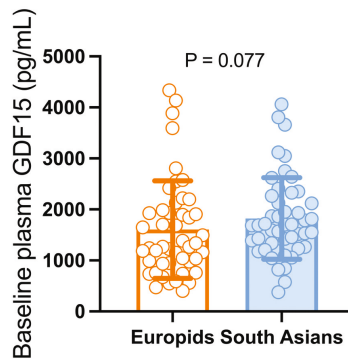
Figure 1. Comparison of plasma GDF15 levels in Europids and South Asians at baseline.
Box plots showing plasma GDF15 levels in Europids (n=47; orange box with circles) compared to South Asians (n=47; blue box with circles) at baseline combined for the placebo and liraglutide treatment groups. Dots represent individual values, boxes represent means, and deviations represent standard deviation (SD).
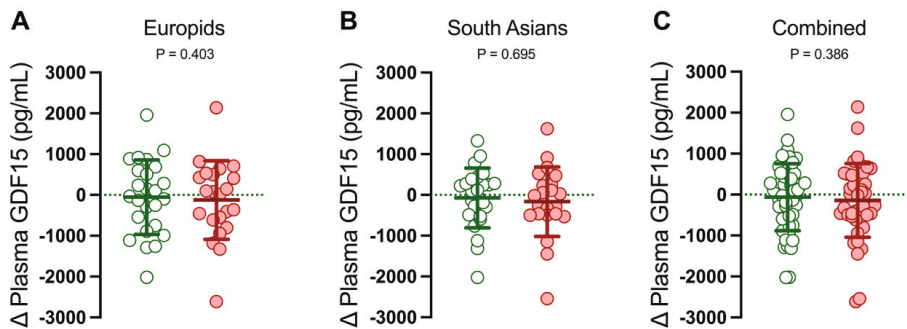
Figure 2. Changes in plasma GDF15 levels in Europids, South Asians, and both ethnicities combined after 26 weeks of placebo or liraglutide treatment.
Scatter plots showing changes in plasma GDF15 (26-week treatment minus baseline values) of (**A**) Europids, (**B**) South Asians, and (**C**) both ethnicities combined after treatment with placebo (n=50; green box and circles) or liraglutide (n=44; red box and circles). Dots represent individual values, horizontal lines represent means, and deviations represent standard deviation (SD).
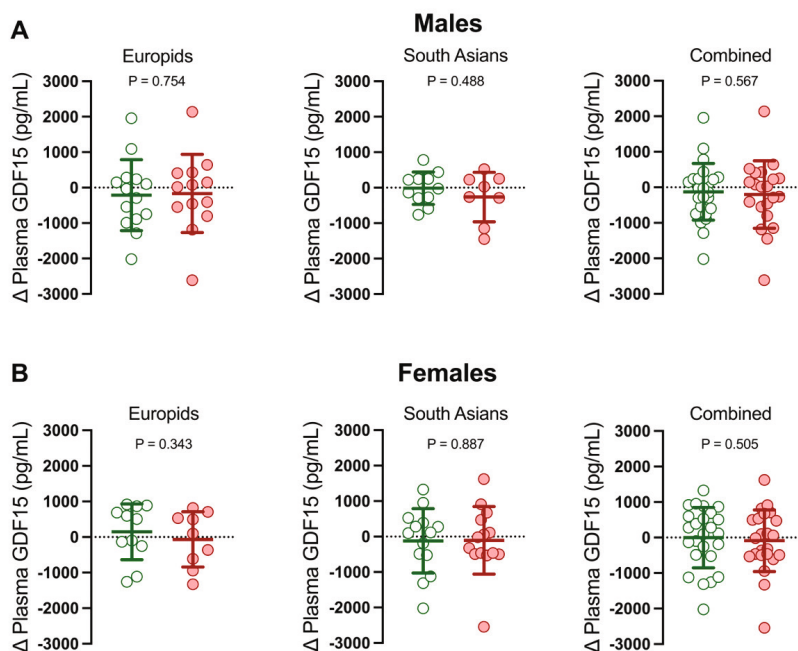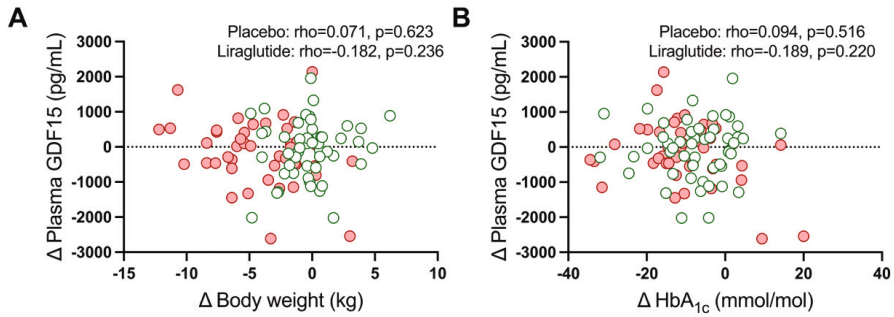
Figure 3. Changes in plasma GDF15 levels in male and female Europids, South Asians, and both ethnicities combined after 26 weeks of placebo or liraglutide treatment

Scatter plots showing changes in plasma GDF15 levels (26-week treatment minus baseline values) of **(A)** Europid males (placebo: n=14; green circles; liraglutide: n=13; red circles), South Asian males placebo: n=11; green circles; liraglutide: n=8; red circles) and both ethnicities combined after placebo (n=25; green circles) or liraglutide (n=21; red circles) and **(B)** Europid females (placebo: n=11; green circles; liraglutide: n=9; red circles), South Asian females (placebo: n=14; green circles; liraglutide: n=14; red circles) of both ethnicities combined after placebo (n=25; green circles) or liraglutide (n=23; red circles). Dots represent individual values, horizontal lines represent means, and deviations represent standard deviation (SD)

Changes in plasma GDF15 levels do not relate to liraglutide-induced decreases in body weight

We next assessed whether the changes in body weight were related to changes in plasma GDF15 levels. Since we did not find any statistical interaction of ethnicity or sex on the changes in plasma GDF15 levels (P ≥ 0.5, data not shown), we pooled all data together to enhance statistical power for the subsequent analyses. After 26 weeks of treatment with liraglutide, as compared to placebo, changes in plasma GDF15 levels were not related to changes in body weight (rho = -0.182; P = 0.236 *vs.* rho = 0.071; P = 0.623, **Fig. 4A**) or changes in HbA$_{1c}$ levels (rho = -0.189; P = 0.220 *vs.* rho = 0.094; P = 0.516 Fig. **4B**). Additionally, we observed that changes in plasma GDF15 levels were not related to changes in blood parameters related to glucose and lipid metabolism (e.g., glucose or total cholesterol) and subcutaneous, visceral, epicardial, paracardial adipose tissue volumes or markers for kidney function (**Figs. 5-7**).
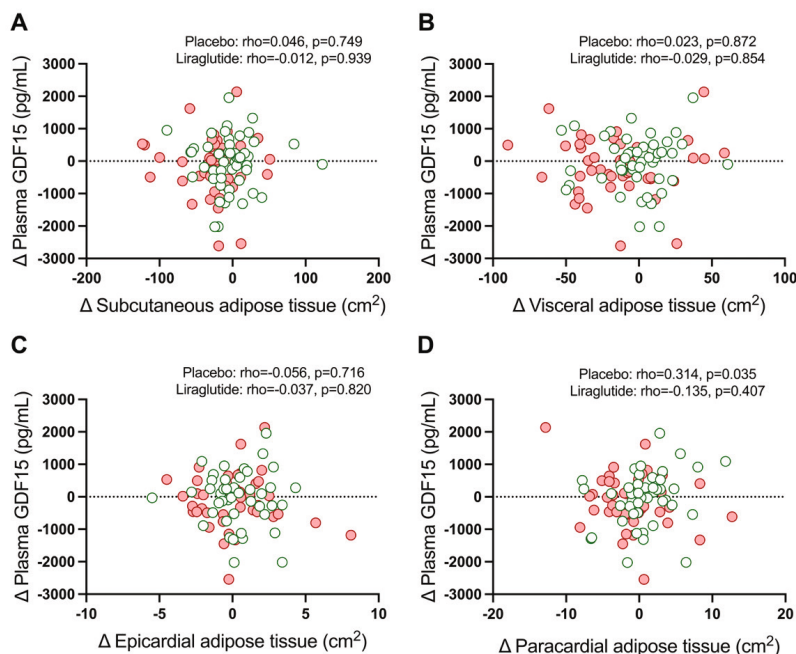
A

B

Figure 4. Correlations between changes in body weight and HbA$_{1c}$ levels and changes in plasma GDF15 levels after 26 weeks of treatment with placebo or liraglutide.
Spearman correlation, in both Europids and South Asians, combined, between the change of body weight and the change in plasma GDF15 levels **(A)** and change in plasma HbA$_{1c}$ levels and change in plasma GDF15 levels after treatment with placebo (n=50; green circles) or liraglutide (n=44; red circles) **(B)**. Dots represent individual values.
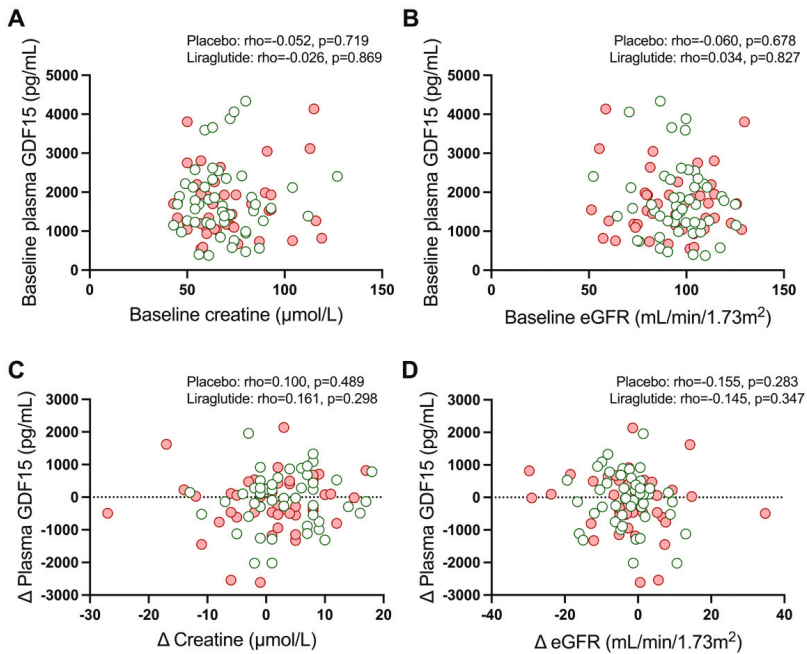
A

B
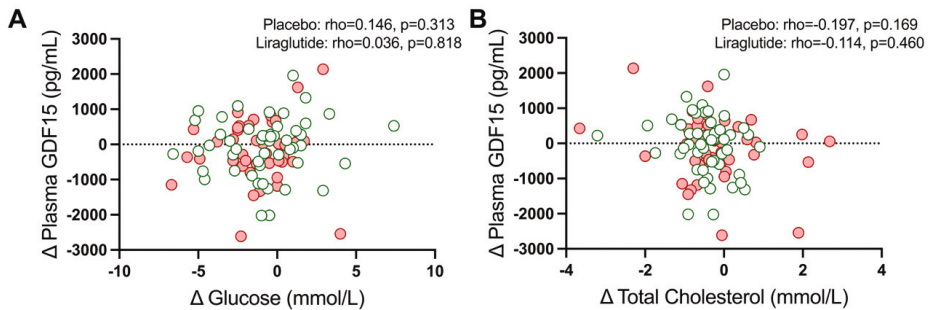
Figure 5. Correlations between changes in metabolic parameters and changes in plasma GDF15 levels after 26 weeks of treatment with placebo or liraglutide
Spearman's correlation plots between changes in fasting plasma glucose and changes in plasma GDF15 levels **(A)** and changes between total cholesterol levels and changes in plasma GDF15 levels **(B)** in both ethnicities combined after treatment with placebo (n=50; green circles) or liraglutide (n=44; red circles). For one participant in the placebo treatment group, the fasting glucose at the end of the study was missing (n=43). Dots represent individual values.

Figure 6. Correlations between changes in subcutaneous adipose tissue, visceral adipose tissue, epicardial adipose tissue, and paracardial adipose tissue volumes with changes in plasma GDF15 levels after 26 weeks of treatment with placebo or liraglutide

Spearman's correlation plots between changes in subcutaneous adipose tissue **(A)**, visceral adipose tissue **(B)**, epicardial adipose tissue **(C)**, or paracardial adipose tissue volumes **(D)** with the change of plasma GDF15 levels in both ethnicities combined after treatment with placebo (n=50; green circles) or liraglutide (n=44; red circles). For six participants in the placebo treatment group, the epicardial adipose tissue (n=44) and for five participants in the placebo group the paracardial adipose tissue (n=45) was not reported. For four participants in the liraglutide treatment group, both epicardial adipose tissue and paracardial adipose tissue volumes could not be reported (n=40) at either the start of the study, at the end of the study, or both. This was due to either unsuccessful ^{1}H-MRS of the heart due to low signal-to-noise ratio incorrect peak frequency, or due to missing data (35, 37). Dots represent individual values.

Figure 7. Correlations between markers for kidney function with plasma GDF15 levels at baseline and after 26 weeks of treatment with placebo or liraglutide
Spearman's correlation plots between baseline serum creatinine levels **(A)** or estimated Glomerular Filtration Rate (eGFR) calculated based on serum creatine levels **(B)** with baseline plasma GDF15 levels in both ethnicities combined. Spearman's correlations between changes in plasma creatine levels **(C)** or changes in eGFR **(D)** with changes in plasma GDF15 levels in both ethnicities combined after placebo (n=50; green circle) or liraglutide (n=44; red circles). Dots represent individual values.

Plasma GDF15 levels do not correlate with metformin doses

As previously published, metformin increases plasma GDF15 levels to suppress appetite, thereby inducing weight reduction (12, 13). Here we found that the metformin dose was not related to plasma GDF15 levels, either at baseline (liraglutide: rho = 0.077; P = 0.617; placebo: rho = 0.071; P = 0.629, **Fig. 8A**) or after 26 weeks of treatment (liraglutide: rho = -0.047; P = 0.762; placebo: rho = -0.004; P = 0.978, **Fig. 8B**).
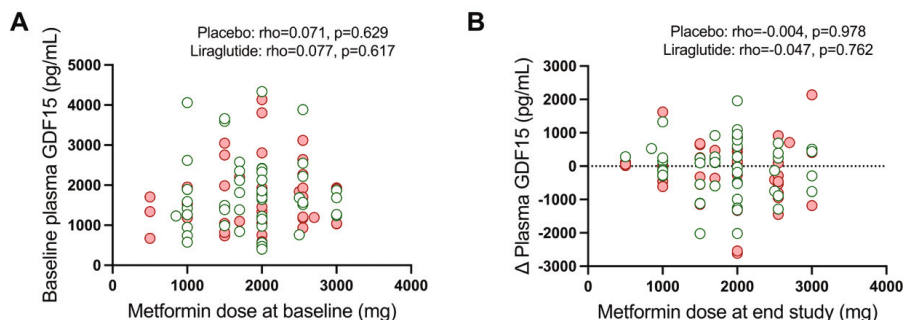
Figure 8. Correlation between metformin dose with change of plasma GDF15
Spearman correlations between the metformin dose at baseline with baseline GDF15 levels **(A)** and between metformin dose at the end of the study with the change in plasma GDF15 levels **(B)** in both ethnicities combined after placebo (n=48; green circles) or liraglutide (n=44; red circles). Two participants in the placebo intervention arm did not use metformin at baseline (placebo; n=48), however, one participant started metformin during the trial (placebo; n=49). Dots represent individual values.

DISCUSSION

The current study showed that plasma GDF15 levels were similar in South Asians compared to Europids with T2DM. Furthermore, 26 weeks of daily treatment with the GLP-1R agonist liraglutide, compared to placebo, did not modify plasma GDF15 levels in both ethnicities. Additionally, we showed that the change in GDF15 levels did not correlate with the decreases in body weight and changes in HbA_{1c} as induced by liraglutide nor with the use of metformin.

In our study, we observed that plasma GDF15 levels at baseline were similar between South Asians compared to Europids. While GDF15 levels have not been previously compared between South Asians and Europids, some studies have investigated GDF15 gene expression and circulating levels between different ethnic groups. For example, one study (38) found that mRNA expression of GDF15 in prostate tissue was lower in African American males compared to American Caucasians who underwent a prostatic biopsy. Another study (39) found that serum GDF15 levels were higher in black people with chronic kidney disease in South Africa compared to the white racial group with chronic kidney disease. Our study specifically focused on differences in plasma GDF15 levels between Europids and South Asians, a population known for their unfavorable metabolic phenotype and high risk of developing cardiometabolic complications (4-6, 40). GDF15 is a well-known biomarker for metabolic diseases, with

higher GDF15 levels corresponding to metabolic diseases such as atherosclerosis, cardiomyopathies, obesity, insulin resistance, and chronic kidney diseases (41-43). Considering the disadvantageous metabolic phenotype of the South Asian population in addition to the previously found differences in the regulation of appetite-regulating hormones, we hypothesized that GDF15 levels would be different in the South Asian population compared to the Europids. However, we did not find a significant difference in plasma GDF15 levels between South Asians and Europids. A possible explanation for this could be the longer duration of diabetes in South Asians. Metformin is the first-line pharmacological intervention for patients with T2DM (44). Since the South Asian participants in this study had longer T2DM duration than the Europid participants, it is possible that they have used metformin for a longer time than Europids, influencing GDF15 levels in this study. Additionally, GDF15 could already have reached a plateau and did not further increase after liraglutide treatment.

Our finding that 26 weeks of liraglutide treatment did not affect plasma GDF15 levels in either ethnicity suggests that the liraglutide-induced weight reduction is independent of the GDF15/GFRAL system. This vision is in line with a recent preclinical study showing that the absence of GDF15 or GFRAL signaling did not affect the ability of liraglutide to reduce food intake in mice (33). There, it was found that while many GFRAL neurons in the area postrema of the mouse and human hindbrain also contained various amounts of GLP-1R mRNA, the majority of the GLP-1R neurons did not express GFRAL mRNA. When mice were injected with liraglutide subcutaneously, food intake decreased independent of whether the mice lacked GFRAL or GDF15. In addition, when mice that lacked the GLP1-R on a whole-body level were injected subcutaneously with GDF15, their food intake was reduced to the same extent as in mice that did not lack the GLP1R. Of note, when mice were injected with the combination of GDF15 and liraglutide, they had a higher reduction of food intake and weight loss than injection of either treatment alone, indicating an independent effect of liraglutide on the GDF15/GFRAL system with possible synergetic potential. Furthermore, our data align with a recent human study that appeared during the preparation of our manuscript (45). In that study, GDF15 levels in male and female patients with obesity (n=20) did not change upon treatment with liraglutide for 5 weeks. Since a previous study involving metformin (12) showed that plasma GDF15 levels continued to rise after a more extended period of metformin treatment (26, 52, and 78 weeks), 5 weeks of treatment with liraglutide may have been too short to modify plasma GDF15 levels. However, in the current study, in which participants were treated for 26 weeks, plasma GDF15 levels also remained unaffected, demonstrating that an extended treatment period of liraglutide treatment is unable to modify plasma GDF15 levels as well. In addition, we did not find a correlation between the change in GDF15 levels upon liraglutide treatment and the change in

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weight loss. Altogether, our study supports that the beneficial effects induced by the GLP-1R agonism liraglutide do not involve the GDF15/GFRAL system.

Taken together, we postulate that the weight-reducing effect of liraglutide is likely mediated by other systems. GLP-1 interacts with vagal afferent neurons to transmit gut signals to the hindbrain where it induces satiety, possibly serving as the primary mechanism for satiety induction by GLP-1 receptor agonism (46). This is supported by the attenuated anorexic effect of GLP-1 agonism when the vagal afferents are denervated (47). In addition, other factors such as delayed gastric emptying, influencing fat distribution, and the possible involvement of brown adipose tissue resulting in enhanced thermogenesis may contribute to liraglutide-induced weight loss as well (48-50). We previously found that treating healthy men with the GLP-1R agonist exenatide for 12 weeks enhanced glucose uptake by brown adipose tissue, pointing to enhanced brown adipose tissue volume (48). Although our current study points towards an effect of liraglutide on weight loss independent of GDF15, the concept of GDF15 as a potential treatment strategy for people living with obesity remains intriguing. A recent study showed that a long-acting GDF15 analogue was successful in reducing food intake in rodents and in humans living with overweight and obesity (22).

Although metformin has previously been shown to increase plasma GDF15 levels, in the current study, we did not find a correlation between the dose of metformin and plasma GDF15 levels, not at baseline nor the end of the intervention period. Although multiple research groups previously described that metformin decreases food intake via increasing plasma GDF15 levels, GDF15 levels were measured up to only 18 months of treatment (12, 13). The participants in our study had been living with diabetes ranging from 11-19 years. To our knowledge, no long-term studies have studied GDF15 levels during prolonged metformin use. Therefore, the GDF15 levels may have reached a plateau or even decreased after long-term treatment, which could explain why we did not find a correlation between metformin dose and plasma GDF15 levels. In addition, the majority of patients included in the study had T2DM for over 10 years and used other pharmacological therapies, such as insulin and/or sulfonylurea derivates, in addition to metformin. Thus, the presence of polypharmacy resulting from prolonged diabetes duration may also have influenced plasma GDF15 levels in our study. In addition, diabetes-related complications, such as atherosclerosis development, as well as the presence of obesity itself may have influenced GDF15 levels.

The strengths of our study are the large sample size of 94 participants, with about 50% females. Secondly, we also included patients of South Asian descent, an ethnic population with a high cardiometabolic disease risk to assess whether ethnic differences

exist in plasma GDF15 levels. Moreover, this would give us the opportunity to assess whether liraglutide-induced effects on GDF15 levels differed between ethnicities. However, this study is not without limitations. We only measured plasma GDF15 levels at two time points: baseline and after 26 weeks of intervention. Therefore, any changes in plasma GDF15 levels within that period could have been missed. Plasma GDF15 is known to be influenced by many factors, such as pharmacological agents (51, 52). Almost half of our population received multiple pharmaceutical agents during the intervention period, which could have influenced plasma GDF15 values as well.

In conclusion, this study showed that plasma GDF15 levels were similar in Dutch South Asians and Dutch Europids. Additionally, we observed that 26 weeks of liraglutide treatment does not modify plasma GDF15 levels in both ethnicities. Therefore, we conclude that the GDF15/GFRAL system likely does not play a role in the weight loss induced by liraglutide.

Acknowledgements
The authors thank the participants of this study.

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