






ORIGINAL ARTICLE

The effects of empagliflozin, dietary energy restriction, or both on appetite-regulatory gut peptides in individuals with type 2 diabetes and overweight or obesity: The SEESAW randomized, double-blind, placebo-controlled trial

Jack A. Sargeant PhD^{1,2}  | James A. King PhD^{2,3}  | Thomas Yates PhD^{1,2} |
 Emma L. Redman PhD^{1,2,4} | Danielle H. Bodicoat PhD⁵ | Sudesna Chatterjee MD⁶ |
 Charlotte L. Edwardson PhD^{1,2} | Laura J. Gray PhD⁷ | Benoit Poulin PhD^{1,2} |
 Ghazala Waheed MSc^{1,2}  | Helen L. Waller PhD^{1,2} | David R. Webb PhD^{1,2,4}  |
 Scott A. Willis PhD^{2,3} | John P. H. Wilding DM⁸  | Kamlesh Khunti PhD^{1,4,9}  |
 David J. Stensel PhD^{2,3,10} | Melanie J. Davies MD^{1,2,4} 

¹Diabetes Research Centre, University of Leicester, Leicester, UK

²National Institute for Health Research (NIHR) Leicester Biomedical Research Centre, Leicester, UK

³School of Sport, Exercise and Health Sciences, Loughborough University, Leicestershire, UK

⁴Leicester Diabetes Centre, University Hospitals of Leicester NHS Trust, Leicester, UK

⁵Simplified Data, Leicester, UK

⁶Milton Keynes University Hospital, Milton Keynes, UK

⁷Department of Health Sciences, University of Leicester, Leicester, UK

⁸Department of Cardiovascular and Metabolic Medicine, University of Liverpool, Liverpool, UK

⁹NIHR Applied Research Collaboration East Midlands, Leicester, UK

¹⁰Faculty of Sport Sciences, Waseda University, Tokorozawa, Japan

Correspondence

Jack A. Sargeant, PhD, Diabetes Research Centre, University of Leicester, Leicester General Hospital, Leicester, LE5 4PW, UK.
 Email: jack.sargeant@uhl-tr.nhs.uk

Abstract

Aim: To assess the impact of the sodium-glucose co-transporter-2 (SGLT2) inhibitor empagliflozin (25 mg once-daily), dietary energy restriction, or both combined, on circulating appetite-regulatory peptides in people with type 2 diabetes (T2D) and overweight or obesity.

Materials and Methods: In a double-blind, placebo-controlled trial, 68 adults (aged 30–75 years) with T2D (drug naïve or on metformin monotherapy; HbA1c 6.0%–10.0% [42–86 mmol/mol]) and body mass index of 25 kg/m² or higher were randomized to (a) placebo only, (b) placebo plus diet, (c) empagliflozin only or (d) empagliflozin plus diet for 24 weeks. Dietary energy restriction matched the estimated energy deficit elicited by SGLT2 inhibitor therapy through urinary glucose excretion (~360 kcal/day). The primary outcome was change in postprandial circulating total peptide-YY (PYY) during a 3-hour mixed-meal tolerance test from baseline to 24 weeks. Postprandial total glucagon-like peptide-1 (GLP-1), acylated ghrelin and subjective appetite perceptions formed secondary outcomes, along with other key components of energy balance.

Results: The mean weight loss in each group at 24 weeks was 0.44, 1.91, 2.22 and 5.74 kg, respectively. The change from baseline to 24 weeks in postprandial total PYY was similar between experimental groups and placebo only (mean difference [95% CI]: −8.6 [−28.6 to 11.4], 13.4 [−6.1 to 33.0] and 1.0 [−18.0 to 19.9] pg/ml in placebo-plus diet, empagliflozin-only and empagliflozin-plus-diet groups, respectively

Jack A. Sargeant and James A. King are joint first authors.

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[all $P \geq .18$]). Similarly, there was no consistent pattern of difference between groups for postprandial total GLP-1, acylated ghrelin and subjective appetite perceptions.

Conclusions: In people with T2D and overweight or obesity, changes in postprandial appetite-regulatory gut peptides may not underpin the less than predicted weight loss observed with empagliflozin therapy.

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KEYWORDS

compensation, energy balance, energy restriction, gut hormones, SGLT2 inhibitors

1 | INTRODUCTION

Sodium-glucose co-transporter-2 inhibitors (SGLT2is) are a class of glucose-lowering therapy in type 2 diabetes (T2D) that promote glucosuria,¹ with the energy deficit resulting from increased urinary glucose excretion (~300-360 kcal/day) underpinning their weight-lowering properties.^{2,3} However, the weight loss observed in both clinical trials and observational studies is 50%-75% less than predicted based on modelling urinary glucose excretion alone.²

Reduced resting energy expenditure (EE) and increased appetite are well-established responses to weight loss in humans after diet or bariatric surgery,⁴ and evidence indicates that compensatory eating may play a key role in attenuating SGLT2i-induced weight loss.⁵ Specifically, a modelling study in humans with T2D estimated that a 13% increase in energy intake (EI) partly explained a lower-than-predicted weight loss (~8 kg) observed after 90 days of empagliflozin therapy.⁶ A similar analysis, following 52 weeks of canagliflozin therapy, suggested that EI increased by ~100 kcal/day for each kilogram of weight loss achieved.⁷ An uncontrolled prospective study in humans (using data from a self-report food frequency questionnaire) supports these analyses,⁸ along with preclinical evidence reporting a dose-dependent increase in EI after dapagliflozin administration in rats with diet-induced obesity.⁹ In the same rodent study, restricting food provision to that consumed by the placebo group augmented the weight loss elicited by dapagliflozin. The extent to which dietary energy restriction can potentiate the weight loss elicited by SGLT2i therapy in humans remains an important question.⁵

The regulation of EI in humans is complex and underpinned by homeostatic, hedonic and environmental factors.¹⁰ Within contemporary models of appetite control, a network of circulating peptides influence eating behaviour and energy homeostasis on an acute (meal-to-meal) and chronic basis.¹⁰ Specifically, peptide-YY (PYY) and glucagon-like peptide-1 (GLP-1) are gut-derived postprandial satiety signals that facilitate efficient nutrient digestion and metabolism,^{11,12} while acylated ghrelin is an orexigenic peptide implicated in the cephalic phase of digestion and meal initiation.^{13,14} These acute signals are moderated by leptin and insulin that inform central appetite circuits about chronic energetic status.^{15,16} Reductions in circulating

leptin and insulin are common responses to weight loss that may encourage compensatory eating,^{3,17,18} although this has recently been debated.¹⁹ Data on the effects of SGLT2i therapies on appetite and appetite-regulatory peptides in humans are sparse, and limited to small and/or uncontrolled studies.²⁰⁻²² Robust evidence from purposive randomized controlled trials is needed to better understand the effects of SGLT2i therapies on appetite and energy balance in individuals with T2D.

The primary aim of this study was to investigate the effects of 24 weeks of empagliflozin therapy, an energy-restricted diet, or both combined, on appetite-regulatory gut peptides in adults with T2D and overweight or obesity, compared with placebo. Effects on subjective appetite perceptions, insulin and leptin, and other components of energy balance, comprised key secondary aims. We hypothesized that empagliflozin would result in an altered profile of appetite-regulatory peptides commensurate with increased perceived appetite (lower total PYY, GLP-1, and higher acylated ghrelin). Moreover, these responses would be greater when combined with an energy-restricted diet, in accordance with greater weight loss.

2 | MATERIALS AND METHODS

2.1 | Study design and participants

SEESAW was a 24-week, randomized, double-blind, placebo-controlled, phase 4 trial conducted at a single clinical research site in Leicester, UK. The trial protocol received full ethical approval from an NHS Research Ethics Committee (16/EM/0040), with all participants giving written, informed consent to participate.

Eligible participants were men or postmenopausal women, aged 30-75 years, with a body mass index (BMI) of 25 kg/m² or higher and T2D controlled through lifestyle advice alone or stable metformin monotherapy (HbA1c 6.0%-10.0% [42-86 mmol/mol]). Key exclusion criteria included individuals with type 1 diabetes, estimated glomerular filtration rate (eGFR) less than 60 ml/min per 1.73 m², familial glucosuria, or other contraindications to SGLT2i therapy. Those prescribed loop diuretics and those consuming a severely

energy-restricted diet (<800 kcal/day) were also excluded. Premenopausal women were excluded given that menstrual cycle phase impacts appetite control and eating behaviour, making standardization problematic.²³ A full list of eligibility criteria is provided in the supplementary methods (see the supporting information).

Participants were randomized (1:1:1:1) using an independent online computerized randomization system (Sealed Envelope Ltd, London, UK), to one of four groups: (a) placebo only, (b) placebo plus energy-restricted diet ("placebo-plus-diet"), (c) empagliflozin 25 mg only ("empagliflozin-only"), or (d) empagliflozin 25 mg plus energy-restricted diet ("empagliflozin-plus-diet"). Randomization was stratified by age (\leq / $>$ 50 years) and BMI ($<$ / \geq 30 kg/m²) and occurred after all baseline measurements.

2.2 | Procedures

Empagliflozin 25 mg or matched placebo were taken orally once daily. Participants unable to tolerate 25 mg of empagliflozin or placebo therapy were withdrawn.

Participants randomized to placebo-plus-diet or empagliflozin-plus-diet groups underwent a personalized energy-restricted diet designed to reduce EI by 360 kcal/day, approximately matching the estimated energy deficit elicited by SGLT2i therapy.^{3,24} Participants received support to identify and maintain a personalized approach to reach a daily EI target, based upon their estimated daily energy requirements (assessed using indirect calorimetry), and accounting for self-reported physical activity. Targets were revised after 6 and 12 weeks.

Participants attended five experimental visits (visits 1-5), occurring at baseline, 2, 6, 12 and 24 weeks. Dietary intake (including alcohol and caffeine) and structured physical activity were standardized before each visit. Upon arrival (08:00 AM-09:00 AM), body mass, BMI, body fat percentage, waist circumference, hip circumference, resting blood pressure and heart rate, and resting EE (indirect calorimetry; GEM open-circuit ventilated hood system, GEM Nutrition Ltd., Cheshire, UK) were measured using standardized procedures.

Approximately 90 minutes into visits 1-5, participants underwent a 3-hour mixed meal tolerance test (MMTT). An intravenous cannula was inserted into an antecubital or forearm vein, after which participants rested for 30 minutes to ensure habituation.²⁵ A fasted blood sample was then collected, before participants consumed a standardized breakfast meal within 15 minutes. Each participant consumed the same meal at each of their visits, containing ~33% of their baseline estimated daily energy requirements and comprising approximately 50% carbohydrate, 15% fat and 35% protein. Further blood samples were collected 30, 60, 90, 120, 150 and 180 minutes after the final mouthful. Subjective appetite perceptions (hunger, fullness, satisfaction and prospective food consumption) were assessed using 100 mm visual analogue scales (VAS) immediately before sample collection.²⁶

Blood samples were drawn into chilled tubes pretreated with an anticoagulant or clotting factor, for the isolation of plasma or serum, respectively. To prevent degradation of acylated ghrelin,

one tube was treated with a protease inhibitor cocktail according to the manufacturer's instructions. Clinical biomarkers were measured using standardized quality-controlled assays within local pathology laboratories. The remaining samples were spun immediately in a refrigerated centrifuge, before plasma/serum were isolated and stored at -80°C for future batch analysis of total PYY, total GLP-1, acylated ghrelin, leptin (all ELISA), insulin, glucagon and C-peptide (multiplex assay). Further details, including assay manufacturer details and coefficients of variation, are included in the supplementary methods (supporting information).

Participants completed the three-factor eating questionnaire (TFEQ)²⁷ and the international physical activity questionnaire (IPAQ; long last 7 days format) at each experimental visit, to assess cognitive dietary restraint, disinhibition and hunger, and self-reported physical activity, respectively. Body composition was assessed at visits 1 and 5 only, using dual-energy X-ray absorptiometry (DEXA; Lunar Prodigy, GE Corporation, CT). Accelerometer-assessed physical activity volume and intensity were captured after visits 0, 3, 4 and 5 (wGT3X-BT, Actigraph, Pensacola, FL). Self-reported daily EI was measured prior to visits 0 and 5 using food diaries.

2.3 | Outcomes

The primary outcome was change from baseline to 24 weeks in postprandial circulating total PYY response during the MMTT. This was assessed via time-averaged total area under the curve (AUC), calculated using the trapezoid method and divided by 3 hours (see supplementary methods [supporting information] for further details). Time-averaged AUC values represent the average concentration across the postprandial period.^{28,29}

Secondary outcomes were change in postprandial total PYY response at 2, 6 and 12 weeks, and change in the following outcomes at 2, 6, 12 and 24 weeks: postprandial responses of total GLP-1, acylated ghrelin, subjective appetite perceptions, glucose, insulin, C-peptide and glucagon; fasting concentrations of leptin (absolute and normalized to body weight), glucose and insulin; TFEQ dimensions; HbA1c; body weight; DEXA-derived body composition (24 weeks only); total body fat percentage (bioelectrical impedance analysis); waist and hip circumferences; daily EI (24 weeks only); resting EE; accelerometer-assessed (6, 12 and 24 weeks only) and self-report (IPAQ) physical activity and sedentary behaviour; C-reactive protein; fasting lipids; eGFR; alanine aminotransferase; and resting blood pressure and heart rate.

Change in all of the above across the follow-up period collectively (analysed using generalized estimating equations [GEE]; see below) also comprised secondary outcomes.

2.4 | Sample size

We required 15 participants per group to complete all trial procedures, to detect a 120 pg/ml difference in postprandial total PYY

between experimental groups and placebo-only with 80% power, assuming a SD of 96.2 pg/ml and a two-sided alpha error rate of 1.7% ($P < .017$).³⁰ The latter allowed three comparisons (one for each experimental group) against placebo-only.

2.5 | Statistical analysis

The primary analysis compared change from baseline in postprandial total PYY at 24 weeks in each experimental group versus placebo-only, using a generalized linear model (GLM) adjusted for age, BMI and baseline total PYY response, utilizing a complete cases approach. Two sensitivity analyses of the primary outcome were performed, repeating the primary analysis in both “intention-to-treat” (ITT) and “per protocol” populations (see supplementary methods [supporting information] for details).

Secondary outcomes were analysed using GLMs as outlined above at 2, 6, 12 and/or 24 weeks (as appropriate), with analyses of accelerometer-measured outcomes additionally adjusted for device wear time. GEE were performed for each outcome to complement GLM analyses at each time point. GEE account for repeated measurements, do not require imputation or exclusion of participants for missing data, and can be interpreted as a “summary” intervention effect across the entire follow-up period. GEE analyses compared each experimental group with placebo-only, using a normal distribution and an exchangeable correlation matrix, and were adjusted as per GLMs. Statistical analyses were performed using STATA v. 16.1 (StataCorp LP, TX).

Comparisons between experimental groups and placebo-only are reported as adjusted mean difference (experimental group minus placebo-only) with 95% CI. To account for multiple testing, statistical significance of all comparisons was determined using Holm's sequential Bonferroni procedure, whereby the three P values for comparisons within a given analysis were assessed sequentially, from lowest to highest, against thresholds of 0.017, 0.025 and 0.050, respectively, stopping once a P value was greater than the threshold to which it was being compared. Further details of statistical analyses can be found in the supplementary methods (supporting information).

3 | RESULTS

3.1 | Participant flow and baseline characteristics

Participant flow is outlined in Figure 1. Recruitment took place from 4 January 2017 to 16 January 2019. Of 68 randomized participants, 63 completed the trial. Within-group change and between-group analyses for all trial outcomes are provided in Tables S2–S4. Those related to appetite regulation, energy balance and glycaemic control are presented in detail below.

Baseline participant characteristics for each group are presented in Table 1 (see Table S1 for combined population). Participants had a median age of 63 years, diabetes duration of 6 years, HbA1c of 6.9% (52 mmol/mol) and BMI of 31.8 kg/m². Thirty-five percent were female, and 82%

were receiving metformin monotherapy for the management of their diabetes. Characteristics were similar across groups, except that there was a greater proportion of female participants in the groups receiving empagliflozin, a lower proportion of individuals receiving metformin monotherapy in the groups receiving the energy-restricted diet, and median diabetes duration was 2–3 years shorter in the placebo-plus-diet group.

3.2 | Body weight changes

The mean weight loss from baseline to 24 weeks was 0.44, 1.91, 2.22 and 5.74 kg in the placebo-only, placebo-plus-diet, empagliflozin-only and empagliflozin-plus-diet groups, respectively. Compared with placebo-only, body weight was lower across individual time points and follow-up collectively in both of the empagliflozin groups, except in the empagliflozin-only group at 24 weeks (which did not reach statistical significance after correction for multiple comparisons). In the placebo-plus-diet group, body weight was statistically lower than placebo-only at 6 weeks (Figure 2A, Tables 2 and S3).

3.3 | Appetite-regulatory peptides

Primary outcome data at baseline and 24 weeks were available for 61 participants, who thus comprised the complete cases population for the primary analysis (Figure 1). There were no differences in postprandial total PYY at 24 weeks between experimental groups and placebo-only (Figure 3A, Table 2). Postprandial total PYY was higher at 12 weeks in the empagliflozin-only group compared with placebo-only, but was otherwise similar between groups at 2, 6 and 12 weeks and across the follow-up period collectively. Results in the ITT population were similar to the primary analysis. However, in the per protocol analysis (placebo-only $n = 11$, placebo-plus-diet $n = 8$, empagliflozin-only $n = 12$, empagliflozin-plus-diet $n = 13$), postprandial total PYY was higher at 24 weeks in the empagliflozin-only group compared with placebo-only (Figure 3A, Tables 2 and S2).

There were no differences in postprandial total GLP-1 or acylated ghrelin between experimental groups and placebo-only at 24 weeks or any intermediate time point (Figure 3B,C, Tables 2 and S2). However, across follow-up collectively, postprandial total GLP-1 was higher in the empagliflozin-only group. Fasting leptin was no different between experimental groups and placebo-only at 24 weeks and intermediate time points, but was lower across follow-up collectively in the empagliflozin-plus-diet group (Figure 3D, Tables 2 and S2). This difference was attenuated and no longer statistically significant when leptin concentrations were normalized to body weight.

3.4 | Subjective appetite perceptions and self-reported EI

There were no differences in VAS-derived appetite perceptions, TFEQ-measured disinhibition and hunger, or self-reported daily EI

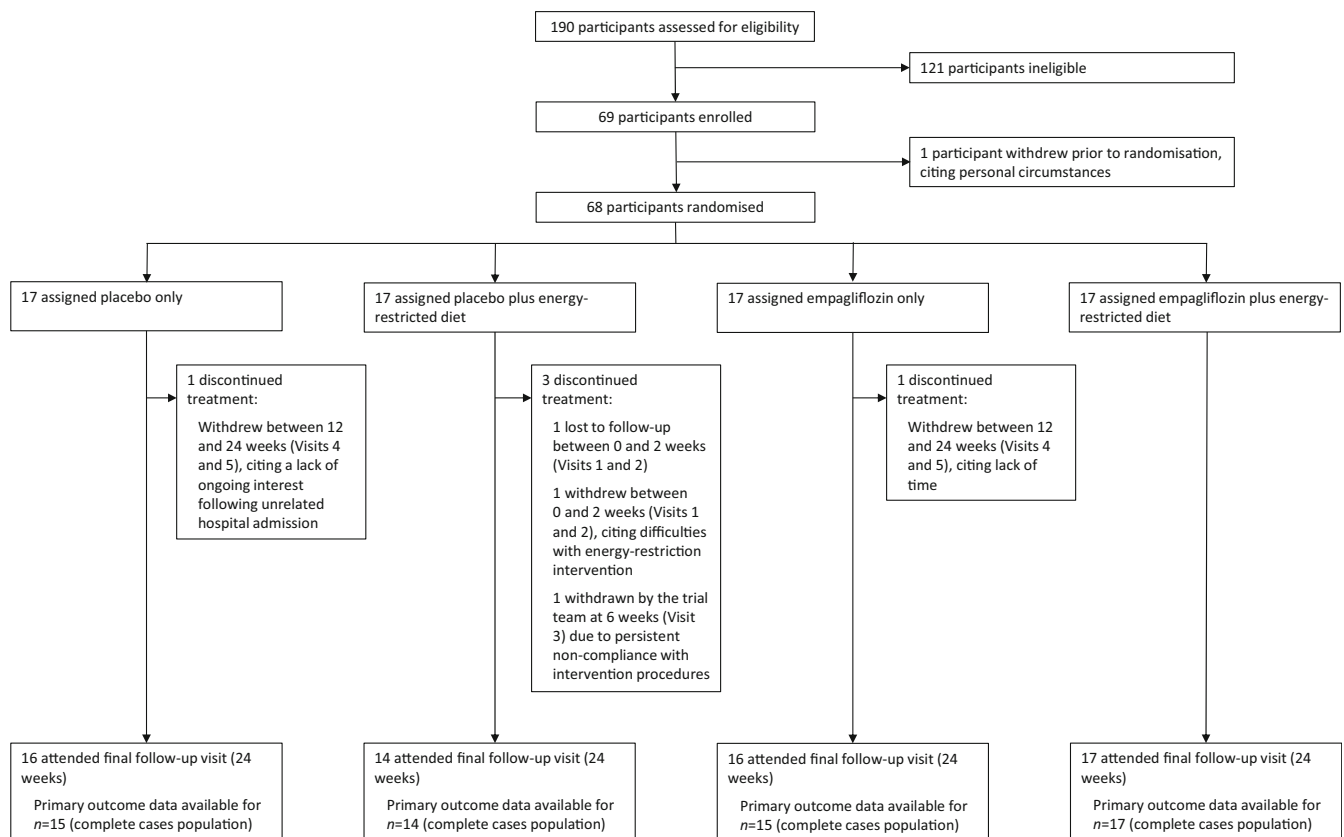


FIGURE 1 Study consort diagram

between experimental groups and placebo-only at any time point or across follow-up collectively (Figure S1A-D, Tables 2 and S2). However, cognitive dietary restraint was higher in the placebo-plus-diet and empagliflozin-plus-diet groups (significantly different in the empagliflozin-plus-diet group at 24 weeks and in both groups at 12 weeks and across follow-up collectively; Tables 2 and S2).

3.5 | Body composition and EE

At 24 weeks, DEXA-derived total body fat mass was lower in the empagliflozin-plus-diet group compared with placebo-only, while total lean body mass was lower in both the empagliflozin-only and empagliflozin-plus-diet groups (Tables 2 and S3). Resting EE was lower at 24 weeks in the empagliflozin-plus-diet group compared with placebo-only (Figure 2B), but daily steps (Figure 2C), sedentary time, and light- and moderate-to-vigorous-intensity physical activity were similar in each experimental group versus placebo-only in all analyses (Tables 2 and S3).

3.6 | Glycaemic control

Compared with placebo-only, HbA1c was lower at 24 weeks in the empagliflozin-plus-diet group, and lower in both empagliflozin-only and empagliflozin-plus-diet groups at 6 and 12 weeks and across

follow-up collectively (Figure 2D, Tables 2 and S4). It was lower in the placebo-plus-diet group at 2 and 6 weeks only. Fasted and postprandial glucose concentrations were lower accordingly (see Tables 2 and S4 for details). Fasting plasma insulin was similar between experimental groups and placebo-only in all analyses performed, but postprandial insulin responses were lower at 24 weeks in all three experimental groups, as well as across follow-up collectively in the empagliflozin-only group (Tables 2 and S4). Postprandial glucagon responses were similar between experimental groups in all analyses.

3.7 | Adverse events

Adverse event data are provided in Table S5. The total number of adverse events and the number of participants with at least one adverse event were similar between groups. Two serious adverse events occurred during the trial (one in each of the placebo-only and placebo-plus-diet groups), but neither were considered related to trial/intervention procedures and neither were fatal.

4 | DISCUSSION

The SEESAW trial investigated the effects of empagliflozin therapy, an energy-restricted diet, and their combination, on appetite

TABLE 1 Participant characteristics at baseline in each group

	Placebo only (n = 17)	Placebo + diet (n = 17)	Empagliflozin only (n = 17)	Empagliflozin + diet (n = 17)
Sex				
Male	13 (76.5)	13 (76.5)	8 (47.1)	10 (58.8)
Female	4 (23.5)	4 (23.5)	9 (52.9)	7 (41.2)
Ethnicity				
White European	12 (70.6)	13 (76.5)	12 (70.6)	12 (70.6)
South Asian	3 (17.7)	3 (17.7)	3 (17.7)	4 (23.5)
Other	2 (11.8)	1 (5.9)	2 (11.8)	1 (5.9)
Age (y)	63 (56-69)	63 (60-69)	61 (57-68)	65 (55-69)
Duration of diabetes (y)	7.0 (6.5-8.0)	4.0 (3.0-8.5)	6.0 (2.5-11.0)	6.5 (4.3-12.3)
Diabetes management				
Lifestyle advice only	1 (5.9)	4 (23.5)	2 (11.8)	5 (29.4)
Lifestyle advice plus metformin monotherapy	16 (94.1)	13 (76.5)	15 (88.2)	12 (70.6)
Glycaemic control				
HbA1c				
%	7.0 (6.6-7.4)	6.9 (6.5-7.4)	6.9 (6.5-7.1)	6.8 (6.5-7.0)
mmol/mol	53 (49-57)	52 (48-57)	51 (48-54)	51 (48-53)
Fasting plasma glucose (mmol/L)	6.9 (6.0-8.3)	6.9 (5.7-7.5)	6.4 (5.7-7.1)	6.8 (5.9-7.3)
Fasting plasma insulin (mU/L)	16.5 (10.2-21.5)	13.2 (10.2-23.3)	17.0 (11.3-23.8)	12.7 (8.6-16.2)
Fasting plasma glucagon (pg/ml)	62 (49-103)	54 (39-84)	58 (33-92)	44 (35-76)
Fasting plasma C-peptide (pg/ml)	1750 (1636-2248)	1689 (1262-2471)	1668 (1279-2437)	1442 (840-1676)
Anthropometry and body composition				
Body weight (kg)	98.8 (86.9-107.4)	96.9 (77.9-110.0)	89.8 (75.7-96.2)	90.3 (77.8-102.9)
BMI (kg/m ²)	32.8 (30.0-36.2)	31.9 (28.4-36.8)	31.4 (29.6-34.9)	31.2 (28.8-34.3)
Body fat (%)	32.0 (30.0-36.5)	34.5 (29.0-38.0)	39.0 (29.5-42.5)	32.0 (27.5-42.5)
Total fat mass (kg)	36.3 (29.6-44.6)	37.9 (25.6-42.8)	33.5 (28.9-38.2)	32.4 (28.4-39.3)
Total lean body mass (kg)	56.4 (51.3-62.8)	57.4 (46.4-66.3)	46.0 (39.5-61.6)	53.8 (44.3-58.2)
Total bone mass (kg)	3.24 (2.55-3.48)	3.15 (2.50-3.50)	2.41 (2.16-3.29)	2.79 (2.40-3.24)
Total bone mineral density (g/cm ²)	1.27 (1.21-1.37)	1.24 (1.11-1.36)	1.22 (1.09-1.31)	1.19 (1.13-1.31)
Waist circumference (cm)	113.0 (103.4-118.5)	112.8 (97.0-120.5)	110.0 (101.8-116.8)	108.0 (98.0-117.2)
Hip circumference (cm)	110.0 (104.0-118.5)	109.8 (101.4-117.3)	111.0 (104.5-117.0)	108.6 (102.6-119.5)
Energy balance and habitual physical activity				
Daily energy intake (kcal/d)	1769 (1573-2170)	1674 (1249-1897)	1698 (1335-2139)	1513 (1177-1742)
Resting energy expenditure (kcal/d)	1566 (1213-1854)	1486 (1282-1930)	1466 (1152-1568)	1431 (1302-1623)
Steps (number/d)	5126 (3640-7198)	4747 (3611-7261)	5077 (4494-7912)	5170 (3769-8533)
Sedentary time (min/d)	611 (524-650)	581 (556-658)	567 (500-689)	585 (519-641)
Light-intensity physical activity (min/d)	268 (210-333)	237 (186-290)	308 (240-339)	281 (231-317)
Moderate-to-vigorous-intensity physical activity (min/d)	17 (10-37)	16 (8-37)	15 (11-25)	19 (5-46)
Renal and hepatic function				
eGFR (mL/min per 1.73 m ²)	90 (83-90)	90 (85-90)	90 (88-90)	90 (87-90)
Albumin (g/L)	46 (43-48)	45 (44-48)	46 (44-47)	46 (44-47)

TABLE 1 (Continued)

	Placebo only (n = 17)	Placebo + diet (n = 17)	Empagliflozin only (n = 17)	Empagliflozin + diet (n = 17)
Alkaline phosphatase (U/L)	82 (75-94)	66 (58-83)	76 (65-92)	85 (74-106)
Alanine transaminase (U/L)	26 (20-36)	35 (19-47)	32 (20-54)	27 (20-36)
Bilirubin (μ mol/L)	9 (8-10)	10 (7-13)	10 (8-10)	7 (6-10)
Blood pressure, lipids and other cardiometabolic risk factors				
Systolic blood pressure (mmHg)	123 (112-134)	130 (115-137)	124 (116-132)	116 (111-135)
Diastolic blood pressure (mmHg)	75 (67-78)	73 (69-85)	73 (69-80)	74 (67-78)
Resting heart rate (beats/min)	74 (58-81)	73 (69-79)	71 (64-83)	74 (67-85)
Total cholesterol (mmol/L)	3.6 (3.2-4.3)	4.0 (3.4-4.6)	3.8 (3.4-4.6)	4.0 (3.2-5.0)
HDL (mmol/L)	1.1 (0.9-1.2)	1.2 (1.0-1.4)	1.4 (1.2-1.6)	1.2 (1.0-1.3)
LDL (mmol/L)	1.7 (1.4-2.0)	2.1 (1.6-2.5)	1.9 (1.3-2.4)	1.9 (1.5-3.0)
Triglycerides (mmol/L)	1.76 (1.34-2.54)	1.77 (1.16-2.21)	1.70 (0.95-2.39)	1.43 (1.26-1.79)
Non-esterified fatty acids (mmol/L)	0.42 (0.36-0.62)	0.41 (0.34-0.73)	0.47 (0.35-0.58)	0.48 (0.43-0.78)
Smoking status				
Never smoked	5 (29.4)	8 (47.1)	7 (41.2)	10 (58.8)
Ex-smoker	9 (52.9)	9 (52.9)	9 (52.9)	5 (29.4)
Current smoker	3 (17.7)	0 (0)	1 (5.9)	2 (11.8)
C-reactive protein (mg/L)	5.0 (5.0-6.5)	5.0 (5.0-5.0)	5.0 (5.0-5.0)	5.0 (5.0-5.0)
Appetite-regulatory peptides				
Fasting total PYY (pg/ml)	87 (60-110)	83 (65-108)	74 (59-109)	72 (53-93)
Postprandial total PYY (pg/ml) ^a	125 (93-182)	140 (120-185)	128 (105-198)	133 (91-159)
Fasting acylated ghrelin (pg/ml)	43 (26-85)	34 (28-53)	78 (34-92)	64 (35-92)
Postprandial acylated ghrelin (pg/ml) ^a	20 (13-55)	18 (11-32)	34 (17-51)	27 (17-75)
Fasting total GLP-1 (pmol/L)	32 (22-42)	33 (25-36)	30 (22-48)	30 (25-36)
Postprandial total GLP-1 (pmol/L) ^a	47 (40-51)	42 (38-53)	46 (34-61)	44 (32-51)
Fasting leptin (ng/ml)	15.3 (7.6-32.3)	14.9 (9.4-16.7)	23.0 (11.1-30.3)	10.7 (7.7-36.7)
Three-factor eating questionnaire dimensions				
Cognitive restraint (AU)	25 (22-29)	26 (22-29)	28 (21-32)	29 (23-33)
Disinhibition (AU)	9 (8-14)	14 (8-17)	15 (7-18)	10 (9-14)
Hunger (AU)	10 (8-11)	11 (10-14)	11 (10-16)	10 (8-13)

Note: Continuous and categorical data presented as median (interquartile range) and frequency (%), respectively.

Abbreviations: AU, arbitrary units; BMI, body mass index; eGFR, estimated glomerular filtration rate; GLP-1, glucagon-like peptide-1; PYY, peptide YY.

^aCalculated as time-averaged area under the concentration-time curve during the standardized 3-hour mixed meal tolerance test.

regulation and key components of energy balance in people with T2D and overweight or obesity. Empagliflozin therapy and matched dietary energy restriction both induced weight loss but did not affect appetite regulatory hormones, appetite perceptions, habitual EI or EE. The combination of interventions potentiated weight loss, alongside reduced circulating leptin concentrations and resting EE, but appetite-regulatory gut peptides, appetite perceptions and EI remained unaffected. Glycaemic control was improved in both groups receiving empagliflozin therapy.

Evidence from murine experiments and human clinical trials indicates that the weight loss elicited by SGLT2i therapies is less than that predicted based on urinary glucose excretion.^{6,7,9} It has been suggested that increased EI, underpinned by enhanced appetite, contributes to this discrepancy,⁶⁻⁹ but this has not been

comprehensively tested.⁵ The principal objective of the SEESAW trial was to examine the impact of our interventions on the appetite-regulatory peptides PYY, GLP-1 and acylated ghrelin. PYY is a gut peptide released into the circulation after meal ingestion that promotes satiety.¹² PYY is released from L-cells predominantly within the lower intestine,³¹ along with GLP-1, another central acting satiety-related peptide that also modulates glycaemia.³² Conversely, acylated ghrelin is a stomach-derived orexigenic peptide implicated in meal initiation and energy conservation.³³ Previous studies have described compensatory changes in the circulating levels of these peptides after weight loss, where PYY and GLP-1 are reduced, and acylated ghrelin is increased.^{4,34} Consequently, we hypothesized that empagliflozin and diet-induced weight loss would alter the profile of these peptides commensurate with increased hunger and

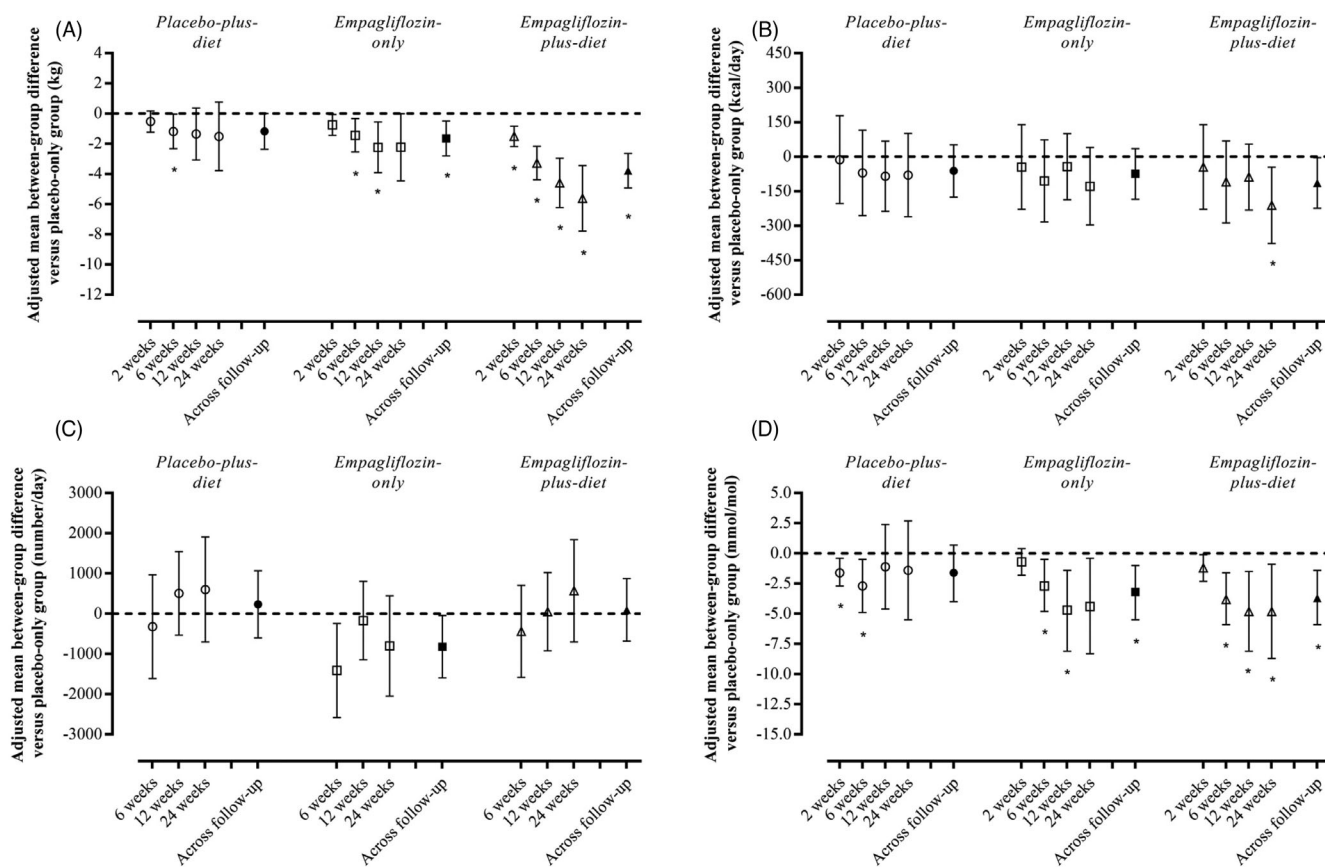


FIGURE 2 Difference between experimental groups and placebo-only at each time point (open symbols) and across follow-up (filled symbols) in A, Total body weight, B, Resting energy expenditure, C, Daily steps and, D, HbA1c. Data are presented as adjusted mean difference (experimental group minus placebo-only) with 95% CI. All analyses were adjusted for age, BMI (both categorized as per randomization) and baseline value of the outcome, with analyses of daily steps additionally adjusted for accelerometer wear time. * Denotes comparison with placebo-only group was statistically significant after application of Holm's sequential Bonferroni procedure to account for multiple comparisons. GEE analyses across follow-up can be inferred as the summary intervention effect. BMI, body mass index; GEE, generalized estimating equations

reduced satiety, with such responses potentiated when the two interventions were combined.

In contrast to our hypothesis, postprandial total PYY was unaffected in our primary analysis (at 24 weeks), and in fact was greater in the empagliflozin-only group within two of our secondary analyses (including our per protocol sensitivity analysis). Similarly, there were no differences between experimental groups and placebo-only in total GLP-1 at 24 weeks, but an increase in the empagliflozin-only group across follow-up collectively. Acylated ghrelin responses were not influenced by any of our interventions. Collectively, these findings indicate that empagliflozin therapy, matched weight loss through dietary energy restriction, and the combination of both do not provoke changes in circulating appetite-related peptides, which would be expected to increase hunger and/or reduce satiety in individuals with T2D and overweight or obesity. It is notable, however, that the magnitude of weight loss elicited in our study is less than that of previous trials within which compensatory responses (increased circulating ghrelin, reduced PYY, and GLP-1) to diet-induced weight loss have

been observed.³⁴ Therefore, changes in these peptides may only become apparent with interventions eliciting greater weight loss. Furthermore, circulating levels of PYY and GLP-1 are reduced in obesity,^{32,35} and thus the capacity for further reduction may be limited. Our documented increase in postprandial GLP-1 concentration in the empagliflozin and empagliflozin-plus-diet treated groups is interesting and may represent a partial normalization of GLP-1 response secondary to weight loss.¹⁹

Working in synergy with acute appetite-regulatory peptides, leptin and insulin are chronic "adiposity signals" that inform the brain about the repleteness of peripheral energy stores.¹⁰ Reductions in circulating leptin and insulin are well characterized responses to weight loss,³⁶ which provoke energy conservation via central effects.³⁷ Across our study, circulating leptin concentrations were reduced in the empagliflozin-plus-diet group, but were unchanged with diet or empagliflozin alone. Despite no change in any experimental group compared with placebo-only at 24 weeks, circulating leptin concentrations were reduced in the empagliflozin-plus-diet group across follow-

TABLE 2 Primary and key secondary outcomes: differences between experimental groups (placebo-plus-diet, empagliflozin-only and empagliflozin-plus-diet) and placebo-only at 24 weeks

	Placebo-plus-diet			Empagliflozin-only			Empagliflozin-plus-diet		
	N	β coefficient (95% CI)	P value	N	β -coefficient (95% CI)	P value	N	β -coefficient (95% CI)	P value
Primary outcome									
Postprandial total PYY (pg/ml) ^a —complete case	14	-8.6 (-28.6 to 11.4)	.400	15	13.4 (-6.1 to 33.0)	.179	17	1.0 (-18.0 to 19.9)	.920
Postprandial total PYY (pg/ml) ^a —ITT	17	-7.3 (-27.2 to 12.5)	.468	17	12.5 (-7.2 to 32.3)	.213	17	1.0 (-18.1 to 20.0)	.921
Postprandial total PYY (pg/ml) ^a —per protocol	8	9.4 (-17.2 to 35.9)	.489	12	29.3 (6.5 to 52.1)	.012	13	7.7 (-15.1 to 30.4)	.510
Secondary outcomes									
Appetite-regulatory peptides, subjective appetite perceptions and three-factor eating questionnaire dimensions									
Postprandial acylated ghrelin (pg/ml) ^a	14	3.2 (-17.7 to 24.1)	.765	15	-13.8 (-34.3 to 6.7)	.187	17	-13.5 (-33.5 to 6.5)	.185
Postprandial total GLP-1 (pmol/l) ^a	14	1.2 (-4.1 to 6.6)	.657	15	3.1 (-2.2 to 8.3)	.250	17	0.3 (-4.8 to 5.5)	.896
Fasting leptin (ng/ml)	14	-1.2 (-5.5 to 3.1)	.588	15	0.6 (-3.6 to 4.9)	.774	17	-4.8 (-8.9 to -0.7)	.022
VAS—Postprandial hunger (mm) ^a	14	-2.6 (-13.4 to 8.2)	.634	14	7.5 (-3.2 to 18.3)	.171	17	3.6 (-6.6 to 13.8)	.488
VAS—Postprandial fullness (mm) ^a	14	3.9 (-8.4 to 16.3)	.534	15	-1.2 (-13.1 to 10.8)	.850	17	-3.5 (-15.1 to 8.1)	.556
VAS—Postprandial satisfaction (mm) ^a	14	5.4 (-7.2 to 17.9)	.401	15	1.3 (-11.0 to 13.6)	.833	17	-5.7 (-17.6 to 6.1)	.344
VAS—Postprandial prospective food consumption (mm) ^a	14	-0.5 (-11.3 to 10.2)	.922	15	3.0 (-7.3 to 13.3)	.564	17	3.1 (-6.9 to 13.1)	.539
TFEQ—Cognitive restraint (AU)	14	2.9 (-0.1 to 5.9)	.056	16	1.4 (-1.5 to 4.3)	.349	17	4.6 (1.7 to 7.5)	.002
TFEQ—Disinhibition (AU)	14	-0.1 (-2.0 to 1.8)	.931	16	0.4 (-1.4 to 2.2)	.679	17	0.6 (1.1 to 2.4)	.475
TFEQ—Hunger (AU)	14	-0.2 (-1.9 to 1.5)	.825	16	0.8 (-0.9 to 2.4)	.367	17	-0.7 (-2.3 to 0.8)	.364
Anthropometry and body composition									
Body weight (kg)	14	-1.52 (-3.79 to 0.76)	.191	16	-2.23 (-4.45 to -0.01)	.049	17	-5.62 (-7.79 to -3.44)	<.001
Total fat mass (kg)	14	-1.94 (-3.72 to -0.16)	.033	16	-0.98 (-2.71 to 0.74)	.264	17	-4.06 (-5.76 to -2.36)	<.001
Total lean body mass (kg)	14	0.34 (-0.50 to 1.18)	.428	16	-1.41 (-2.23 to -0.60)	.001	17	-1.60 (-2.40 to -0.80)	<.001
Energy balance and habitual physical activity									
Daily energy intake (kcal/d)	15	226 (-82 to 535)	.151	14	252 (-56 to 560)	.109	13	210 (-112 to 532)	.202
Resting energy expenditure (kcal/d)	13	-80 (-261 to 101)	.387	16	-129 (-297 to 40)	.134	17	-212 (-378 to -45)	.013
Steps (number/d)	13	604 (-698 to 1906)	.363	15	-800 (-2047 to 447)	.209	14	574 (-695 to 1843)	.375
Sedentary time (min/d)	13	-23 (-65 to 19)	.283	15	1 (-40 to 41)	.974	14	-18 (-59 to 23)	.395
Light-intensity physical activity (min/d)	13	20 (-15 to 56)	.261	15	-2 (-36 to 32)	.909	14	10 (-24 to 45)	.558
Moderate-to-vigorous-intensity physical activity (min/d)	13	3 (-9 to 15)	.586	15	-1 (-13 to 11)	.863	14	7 (-5 to 19)	.249
Glycaemic control									
HbA1c (mmol/mol) ^b	14	-1.4 (-5.5 to 2.7)	.512	16	-4.4 (-8.3 to -0.4)	.032	17	-4.8 (-8.7 to -0.9)	.016
Fasting plasma glucose (mmol/L)	13	-0.05 (-0.63 to 0.53)	.872	16	-0.88 (-1.44 to -0.31)	.002	17	-1.23 (-1.78 to -0.69)	<.001
Postprandial plasma glucose (mmol/L) ^a	14	0.06 (-1.12 to 1.24)	.919	15	-1.24 (-2.40 to -0.08)	.036	17	-1.66 (-2.78 to -0.53)	.004

(Continues)

TABLE 2 (Continued)

	Placebo-plus-diet			Empagliflozin-only			Empagliflozin-plus-diet		
	N	β coefficient (95% CI)	P value	N	β -coefficient (95% CI)	P value	N	β -coefficient (95% CI)	P value
Fasting plasma insulin (mU/L)	14	-1.2 (-5.7 to 3.3)	.613	15	-4.4 (-8.7 to 0.0)	.048	17	-4.3 (-8.6 to 0.0)	.048
Postprandial plasma insulin (mU/L) ^a	14	-26.8 (-45.7 to -7.9)	.006	15	-32.3 (-51.4 to -13.3)	.001	17	-20.9 (-38.9 to -2.9)	.023
Postprandial glucagon (pg/ml) ^a	14	-5.5 (-20.1 to 9.1)	.461	15	-4.6 (-18.9 to 9.7)	.530	17	-6.5 (-20.4 to 7.5)	.363

Notes: Analyses conducted using a complete-case approach unless otherwise specified, with generalized linear models adjusted for age, BMI (both categorized as per randomization) and baseline value of the outcome; P values in bold are statistically significant (assessment of statistical significance used the Holm sequential Bonferroni procedure to account for multiple comparisons).

Abbreviations: AU, arbitrary units; BMI, body mass index; GLP-1, glucagon-like peptide-1; ITT, intention-to-treat; PPY, peptide YY; TFEQ, three-factor eating questionnaire; VAS, visual analogue scale.

^aCalculated as time-averaged area under the concentration-time curve during the standardized 3-hour mixed meal tolerance test.

^bStatistical analyses of HbA1c were performed on data in mmol/mol only. Converted values of within-group change and between-group differences in % can be found in Table S4.

up collectively. While fasting insulin concentrations remained unchanged, insulin responses to the MMTT were reduced at 24 weeks in the empagliflozin-only and empagliflozin-plus-diet groups. Our data are consistent with a recent meta-analysis showing reduced circulating leptin concentrations in people with T2D following therapy with another SGLT2i (ipragliflozin),¹⁸ while reductions in circulating insulin in response to SGLT2i therapy (including empagliflozin) have previously been documented in mechanistic experiments.^{3,17,24} Reductions in postprandial insulinaemia in the empagliflozin groups probably occurred secondary to improvements in glycaemia,¹⁷ whereas the greater weight (fat mass) loss probably explains why leptin was reduced only in the empagliflozin-plus-diet group. The superior weight loss with the combination of interventions may also explain why resting EE was lower only in the empagliflozin-plus-diet group.

Another notable finding of our study is that subjective appetite perceptions were also unresponsive to each of the interventions. This suggests that the energy deficits elicited by empagliflozin therapy, dietary energy restriction, and their combination, did not provoke physiological responses reaching the level of consciousness in our participants. This finding contrasts previous data showing increased subjective appetite (particularly an increase in fasting and meal-related hunger) in response to negative energy balance and weight loss,³⁶ including a single, uncontrolled human study in individuals with T2D undergoing ipragliflozin therapy.²² These discrepant findings may be explained by the modest magnitude of weight loss elicited in our study (particularly in the placebo-plus-diet and empagliflozin-only groups; ~3% to 3.5% from baseline), or it may be that, despite exhibiting best practice, our assessment of appetite within a laboratory environment lacked sufficient sensitivity to detect subtle changes in subjective perceptions. It is also noteworthy that we observed no increase in self-reported EI in any experimental group compared with placebo. These findings contrast evidence from preclinical studies in rodents and previous modelling analyses in humans,^{6,8,9} but are in accordance with preliminary analyses from a randomized placebo-controlled crossover trial in people with T2D, which reports no change in laboratory-assessed food intake following 12 weeks of dapagliflozin therapy.^{38,39}

This study was the first to assess the impact of SGLT2i therapy on habitual physical activity, which is the most variable component of total daily EE. While our results do not suggest a conclusive effect (no statistically significant differences were apparent after adjustment for multiple comparisons), the nominally lower number of steps per day across follow-up in the empagliflozin-only group (~800 steps) is noteworthy and supportive data in rodents exist. In a study of mice undergoing canagliflozin therapy alongside a high-fat diet, daily wheel running was reduced compared with those fed a high-fat diet alone.⁴⁰ Potential changes in habitual physical activity after initiating SGLT2i therapy thus requires further investigation, particularly given the low habitual activity in our study population (~5000 steps/day), and that as little as 500 steps/day change in activity has been postulated as clinically meaningful.⁴¹

The key strengths of this trial include the precision in matching within-group weight change in the placebo-plus-diet and empagliflozin-

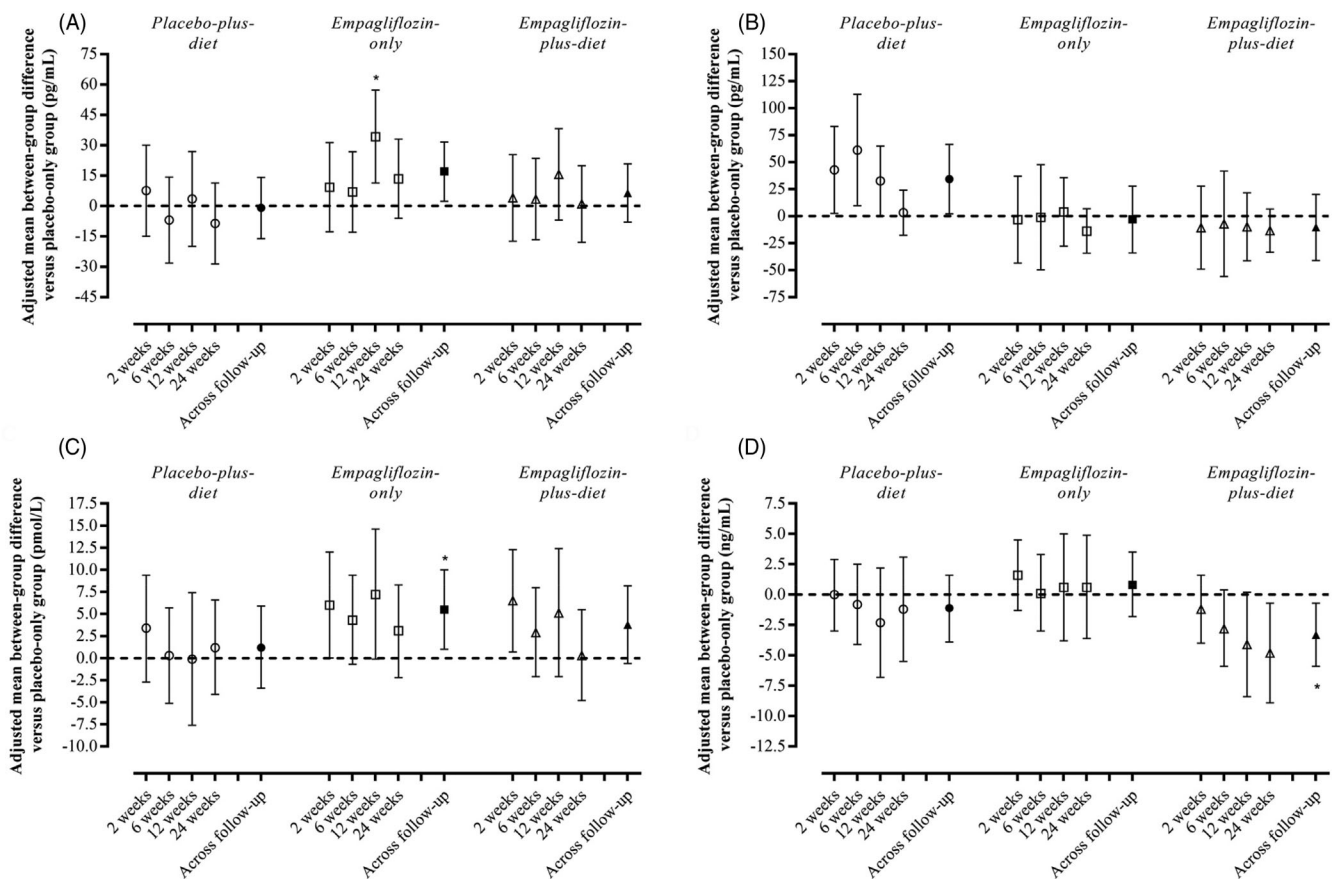


FIGURE 3 Difference between experimental groups and placebo-only at each time point (open symbols) and across follow-up (filled symbols) in appetite-regulatory gut peptides: A, Postprandial total PYY, B, Postprandial acylated ghrelin, C, Postprandial total GLP-1, and D, Fasting leptin. Data are presented as adjusted mean difference (experimental group minus placebo-only) with 95% CI. Postprandial responses were assessed as time-averaged area under the concentration-time curve during the standardized 3-hour mixed meal tolerance test. All analyses were adjusted for age, BMI (both categorized as per randomization) and baseline value of the outcome. * Denotes comparison with placebo-only group was statistically significant after application of Holm's sequential Bonferroni procedure to account for multiple comparisons. GEE analyses across follow-up can be inferred as the summary intervention effect. BMI, body mass index; GEE, generalized estimating equations; GLP-1, glucagon-like peptide-1; PYY, peptide YY

only groups (−3.6% and −3.2%, respectively), as well as the detailed assessment of appetite regulatory responses to a MMTT and components of EE in a multiethnic population (~28% non-White). Lack of assessment of hedonic mediators of appetite control are a noteworthy limitation that should be investigated in future trials. Larger trials are also needed to examine whether the findings from our tightly controlled experimental design translate into changes in eating behaviour in real-world settings. The possibility that we may have been underpowered to detect small but potentially significant differences in some secondary outcomes should also be acknowledged.

In conclusion, this trial shows that 24 weeks of empagliflozin therapy, matched diet-induced weight loss, or the combination of both, do not provoke consistent changes in appetite regulatory gut peptides (PYY, GLP-1 and acylated ghrelin) or subjective appetite perceptions in individuals with T2D and overweight or obesity. Therefore, changes in these outcomes, at least over 24 weeks, do not underpin potential compensatory increases in EI that have been suggested to attenuate the weight loss elicited with SGLT2i therapy. Future studies should scrutinize the impact of SGLT2i therapies on

eating behaviour and its determinants, including hedonic mediators of appetite control, physical activity and exercise habits, as well as examine the combination of dietary interventions and SGLT2is in real-world clinical practice.

AUTHOR CONTRIBUTIONS

MJD and SC generated the research idea. MJD, JK, TY, ELR, DB, SC, CLE, LJG, DRW, KK and DJS designed the study and/or the statistical analysis plan. JAS, JAK, TY, ELR, SC and DRW oversaw data collection and/or intervention delivery. JAS, JAK, BP, HLW, SAW and DJS were responsible for biochemical analyses. CLE processed the accelerometer data. JAS, TY, DB, LJG and GW were responsible for data analyses. JAS, JAK, TY and MJD interpreted findings and drafted the manuscript. All authors commented on the draft, providing clinical and/or academic input, approved the final version and agree to be accountable for the work. JAS is the guarantor of the work and as such had full access to all the data and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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CONFLICT OF INTEREST

JAS and TY report funding in the form of an investigator-initiated trial from AstraZeneca. DRW has received honoraria as a speaker for AstraZeneca, Sanofi-Aventis and Lilly, and has received research funding support from Novo Nordisk. JPHW reports receiving consultancy fees (paid to University of Liverpool) in relation to obesity and type 2 diabetes in the last 12 months from AstraZeneca, Boehringer Ingelheim, Janssen Pharmaceuticals, Lilly, Mundipharma, Napp, Novo Nordisk, Rhythm Pharmaceuticals, Sanofi and Saniona; personal fees honoraria/lecture fees from AstraZeneca, Boehringer Ingelheim, Lilly, Napp, Mundipharma, Sanofi and Takeda; and research grant funding (via University of Liverpool) from AstraZeneca and Novo Nordisk. KK has acted as consultant, advisory board member and speaker for Abbott, Amgen, Astrazeneca, Bayer, NAPP, Lilly, Merck Sharp and Dohme, Novartis, Novo Nordisk, Roche, Berlin-Chemie AG/Menarini Group, Sanofi-Aventis, Servier and Boehringer Ingelheim; and has received EACME grants from Boehringer Ingelheim, AstraZeneca, Novartis, Novo Nordisk, Sanofi-Aventis, Lilly, Merck Sharp & Dohme. MJD has acted as consultant, advisory board member and speaker for Novo Nordisk, Sanofi, Lilly and Boehringer Ingelheim, an advisory board member and speaker for AstraZeneca, an advisory board member for Janssen, Lexicon, Servier and Gilead Sciences Ltd and as a speaker for Napp Pharmaceuticals, Mitsubishi Tanabe Pharma Corporation and Takeda Pharmaceuticals International Inc. She has received grants in support of investigator and investigator initiated trials from Novo Nordisk, Sanofi-Aventis, Lilly, Boehringer Ingelheim, AstraZeneca and Janssen. JAK, ELR, DHB, SC, CLE, LJG, BP, GW, HLW, SAW and DJS have no conflicts of interest to declare.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/dom.14721>.

DATA AVAILABILITY STATEMENT

The datasets generated during the current study are available from the corresponding author on reasonable request.

ORCID

Jack A. Sargeant  <https://orcid.org/0000-0003-0395-7329>

James A. King  <https://orcid.org/0000-0002-8174-9173>

Ghazala Waheed  <https://orcid.org/0000-0002-5142-110X>

David R. Webb  <https://orcid.org/0000-0002-3932-3339>

John P. H. Wilding  <https://orcid.org/0000-0003-2839-8404>

Kamlesh Khunti  <https://orcid.org/0000-0003-2343-7099>

Melanie J. Davies  <https://orcid.org/0000-0002-9987-9371>

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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