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ORIGINAL ARTICLE

Clinical Trials and Investigations

Glucagon-like peptide-1/glucagon receptor agonism associates with reduced metabolic adaptation and higher fat oxidation: A randomized trial

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Abstract

Objective: This study tested the hypothesis that treatment with the glucagon-like peptide-1/glucagon receptor agonist SAR425899 would lead to a smaller decrease in sleeping metabolic rate (SMR; kilocalories/day) than expected from the loss of lean and fat mass (metabolic adaptation).

Methods: This Phase 1b, double-blind, randomized, placebo-controlled study was conducted at two centers in inpatient metabolic wards. Thirty-five healthy males and females with overweight and obesity (age = 36.5 ± 7.1 years) were randomized to a calorie-reduced diet (−1000 kcal/d) and escalating doses (0.06-0.2 mg/d) of SAR425899 ($n = 17$) or placebo ($n = 18$) for 19 days. SMR was measured by whole-room calorimetry.

Results: Both groups lost weight (−3.68 ± 1.37 kg placebo; −4.83 ± 1.44 kg SAR425899). Those treated with SAR425899 lost more weight, fat mass, and fat free mass ($p < 0.05$) owing to a greater achieved energy deficit than planned. The SAR425899 group had a smaller reduction in body composition-adjusted SMR ($p = 0.002$) as compared with placebo, but not 24-hour energy expenditure. Fat oxidation and ketogenesis increased in both groups, with significantly greater increases with SAR425899 ($p < 0.05$).

Conclusions: SAR425899 led to reduced selective metabolic adaptation and increased lipid oxidation, which are believed to be beneficial for weight loss and weight-loss maintenance.

INTRODUCTION

The incretin hormones glucagon-like peptide-1 (GLP-1) and glucagon have shown promise as weight-loss therapies. GLP-1 regulates nutrient metabolism and decreases food intake. Significant weight loss has been observed with administration of the long acting GLP-1 receptor (GLP-1R) agonists liraglutide [1] and semaglutide [2], primarily by affecting satiety and food intake. Glucagon is another satiety hormone that also regulates nutrient metabolism [3]. Glucagon receptor (GCGR) activation seems to promote weight loss via increased energy expenditure (EE) in preclinical models although the impact in humans is unclear [4–6]. Glucagon's action through GCGR to promote weight loss may be though the inhibition of appetite or reduced food intake [7]. Given the actions of GLP-1 and glucagon on energy intake (EI) and expenditure, dual agonists of GLP-1R and GCGR have been shown to produce weight loss while avoiding the hyperglycemic effects of pure glucagon agonism [6].

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Loss of body energy stores (BES) is primarily driven by the following: a reduction of EI [8, 9] and/or an increase in EE, and/or a reduction in EE that is smaller than expected based on changes in body composition (reduced metabolic adaptation) [8–11]. It is now recommended that next generation weight-loss agents should target not only weight loss, but also a decrease in metabolic adaptation and an increase in fat oxidation (FatOx), thus preventing the loss of fat free mass (FFM) [12]. The contribution of these metabolic parameters to the weight loss induced by the dual activation of GLP-1R and GCGR is unknown.

Therefore, we evaluated effects of a novel dual GLP-1R/GCGR agonist with higher potency for GLP1-R, SAR425899, on energy metabolism such as EE and FatOx [13, 14]. This short duration study implemented the appropriate experimental paradigm to quantify the effect of SAR425899 on 24-hour sleep metabolic rate (SMR; normalized to 24 hours) and substrate oxidation following multiple escalating subcutaneous doses over 19 days. We achieved this by using caloric restriction with the goal of similar changes in weight and body composition in the two groups so that we could adequately evaluate metabolic adaptation. To address metabolic adaptation, we elected to use SMR because it has the lowest interindividual variability as it is not affected by factors such as physical activity, stress, and food consumption [15, 16]. We hypothesized that a program of caloric restriction plus SAR425899, compared with caloric restriction alone, would attenuate the expected fall in SMR (reduced metabolic adaptation) and stimulate FatOx.

Study Importance

What is already known?

- Preclinical studies demonstrate that glucagon alone or in combination with glucagon-like peptide-1 increases energy expenditure and weight loss.
- Short-term studies in humans are equivocal regarding the energy expenditure effects of glucagon.

What does this study add?

- We tested the hypothesis that, during weight loss, a novel dual agonist of GLP-1 and glucagon receptors (SAR425899) would lead to a smaller reduction in sleeping metabolic rate (kilocalories/day) than predicted by changes in body composition during weight loss (metabolic adaptation).
- We found that SAR425899 led to reduced metabolic adaptation, increased fat oxidation, and enhanced ketogenesis independent of the changes in body energy stores.

How might these results change the direction of research?

- After accounting for changes in energy stores, SAR425899 leads to metabolic benefits (lower selective metabolic adaptation and increased fat oxidation) that may be important for weight loss and weight-loss maintenance.
- Pending the development of an ideal combination of agonists that is well tolerated, these metabolic benefits could lead to improved weight loss and longer-term weight-loss maintenance.

METHODS

The details of the study design were previously reported. Herein, we briefly describe these components and provide details for aspects of the study not reported elsewhere [17].

Design

This was a Phase Ib, two-center, double-blind, randomized, placebo-controlled study that enrolled the first participant April 17, 2018, and completed the last participant on December 14, 2018. Treatment duration was 19 days of SAR425899 with once daily dosing in the morning. Dose escalations occurred after 4, 8, and 12 days of treatment after a 7-day run-in period (Supporting Information Figure S1). This trial (NCT03376802) was approved by the Institutional Review Boards of the two study sites: AdventHealth in Orlando, Florida, and Pennington Biomedical Research Center in Baton Rouge, Louisiana. All potential participants provided written informed consent.

Participants

Volunteers were generally healthy, weight stable males and females, had body mass index (BMI) of 28 to 40 kg/m^2, and were 18 to 50 years of age. Inclusion and exclusion criteria are provided in online Supporting Information. Thirty-five participants were randomized. Eighteen participants were randomly allocated to placebo and 17 to SAR425899. Study treatment discontinuation due to adverse events (AEs) or personal reasons occurred for seven participants. This left 17 in the placebo group and 11 in the SAR425899 group for the pharmacodynamic assessments presented herein (Consolidated Standards of Reporting Trials [CONSORT] diagram provided in Supporting Information Figure S2). Demographics, anthropometrics, and site enrollment were similar between study completers and the people who were randomized but who did not complete the study (Supporting Information Table S1).

Randomization and dosing

Participants were randomized 1:1 centrally by the sponsor to the treatment groups (SAR425899/placebo) using site and sex as stratification factors. The following SAR425899 doses were administered subcutaneously: 0.06 mg from Day 1 to 4, 0.12 mg from Day 5 to 8, 0.16 mg from Day 9 to 12, and 0.2 mg from Day 13 to 19. A placebo solution was administered at equivalent volumes. The pen-type injector (TactiPen)

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was provided for each participant separately from the cartridge kits. A double-blind design was implemented with cartridges (SAR425899 or placebo) and pen devices being indistinguishable. Supporting Information Table S2 provides pharmacokinetic data (secondary end point).

Safety and tolerability

To address our secondary objectives of safety and tolerability, AEs were monitored throughout the study based on prior studies [18, 19] via medical history, vital signs, physical examination, and standard laboratory assessments (hematology, biochemistry, coagulation, urinalysis). Safety assessments are detailed in online Supporting Information.

Nutrition intervention

Caloric restriction of the placebo group was necessary because of the confounding effects of metabolic adaptation, which is defined as a decrease in EE greater than predicted by the change in weight and body composition [20–23]. To ensure the achievement of a 1000-kcal deficit and similar weight loss, participants were housed in a metabolic ward during the treatment period [13].

Participants consumed a weight-maintaining outpatient diet until randomization [17]. After baseline measures and randomization, both groups were placed in a 1000-kcal deficit per day [17]. Such a caloric deficit was a priori estimated to result in a reduction of body weight of ∼3 kg and fat mass (FM) of ∼1.6 kg and a decrease in 24-hour EE of ∼350 kcal/d within 3 weeks of treatment in both arms. This amount of weight loss is similar to what has been observed in other studies with SAR425899 [13]. A fixed calorie deficit was selected over other methods (fixed body weight reduction or percentage calorie deficit) based on data from a validated mathematical model of human metabolism [24, 25], suggesting more consistent change of body weight, FFM, and FM in people with disparate body weight. The diet consisted of 15% protein, 30% fat, and 55% carbohydrates; 100% diet consumption was required. Overall adherence (kilocalories presented vs. weighed back unconsumed food) was 94% (Supporting Information Table S3). Supporting Information Table S4 shows a list of allowed

TABLE 1 Demographics and baseline characteristics

	Placebo ($n = 17$)	SAR425899 ($n = 11$)	All	p value
Age (y), mean (SD)	36.1 (7.3)	35.7 (8.6)	36.0 (7.7)	0.896
Sex, *n* (%)				
Female	6 (35.3)	3 (27.3)	9 (32.1)	0.655
Male	11 (64.7)	8 (72.7)	19 (67.9)	
Race, *n* (%)[a]				
White	8 (50.0)	3 (27.3)	11 (40.7)	0.490
Black or African American	6 (37.5)	6 (54.5)	12 (44.4)	
Other[b]	2 (12.5)	2 (18.2)	4 (14.8)	
Ethnicity, *n* (%)				
Hispanic or Latino	7 (41.2)	2 (18.2)	9 (32.1)	0.327
Not Hispanic or Latino	9 (52.9)	7 (63.6)	16 (57.1)	
Not reported	1 (5.9)	2 (18.2)	3 (10.7)	
Weight (kg), mean (SD)	91.54 (12.15)	93.76 (10.10)	92.41(11.24)	0.604
BMI (kg/m^2), mean (SD)	30.33 (2.23)	30.95 (2.55)	30.57 (2.34)	0.521
Fat mass (kg), mean (SD)	33.6 (6.1)	35.4 (6.7)	34.3 (6.3)	0.473
Fat free mass (kg), mean (SD)	57.9 (11.6)	58.3 (9.3)	58.0 (10.5)	0.931
HbA_{1c} (%), mean (SD)	5.477 (0.286)	5.236 (0.505)	5.380 (0.396)	0.173
Glucose (mmol/L), mean (SD)	5.261 (0.500)	5.126 (0.437)	5.208 (0.473)	0.458
Total cholesterol (mmol/L), mean (SD)	4.986 (1.098)	4.759 (0.838)	4.897 (0.993)	0.542
HDL (mmol/L), mean (SD)	1.167 (0.306)	1.246 (0.313)	1.198 (0.305)	0.515
LDL (mmol/L), mean (SD)	3.00 (0.90)	2.55 (0.84)	2.89 (0.79)	0.277
Triglycerides (mmol/L), mean (SD)	1.783 (0.920)	1.947 (2.000)	1.848 (1.411)	0.802

Note: Data presented for participants who completed baseline and post-treatment calorimetry visits (pharmacodynamic population). Variables are shown as mean (SD) or *n* (%). Placebo vs. SAR425899 at baseline. Comparison by unpaired *t* test.
Abbreviations: HbA_{1c}, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
[a]$n = 16$ for placebo group race variable.
[b]Other = unknown, multiple, or Asian.

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and disallowed beverages and seasonings. A sample menu is provided in Supporting Information Table S5 with extended methods in online Supporting Information.

Energy requirements

Run-in period

Energy needs for the outpatient days (Days −7 through −4) were estimated using the following equation (rounded to the nearest 100 kcal) derived from doubly labeled water studies: 24-hour EE (kcal/d) = 1279 + 18.3(weight, kg) + 2.3(age, y) − 338(sex: 1 = female, 0 = male) [17, 26]. Energy requirements for the first calorimetry day (Day −3) were estimated using the following equation derived from calorimeter studies: 24-hour EE (kcal/d) = 26.2(FFM, kg) + 5.2(FM, kg) − 2.32(age, y) − 96 (African American) + 546 [27]. Within-day adjustments, if needed, were made based on software predictions that were input into published calorimetry equations [27]. Between-day adjustments were based on the prior day.

Treatment period

Energy needs for the treatment period (Days 1-19) were estimated using the following equation, assuming a 20% increased EE on the metabolic ward compared with that in the calorimeters: EI = (EE in calorimeter [average Days −3, −2, −1] × 1.2) − 1000 kcal [17].

Whole-body room indirect calorimetry

Standards for calorimetry operations used in our study are described in Chen et al. [16] and calorimetry methods described in Allerton et al. [17]. Briefly, 24-hour EE and related components (SMR: primary end point; secondary end points: resting metabolic rate; basal metabolic rate; thermic effect of food) and substrate oxidation (respiratory exchange ratio [RER]; RER during sleep; RER over a period of 24 hours [$RER_{24\text{-}h}$]; RER during rest, basal RER; oxidation rates [protein, carbohydrate, and fat]) were measured by indirect calorimetry on three consecutive days during the run-in period (Days −3, −2 and −1: baseline) and at the end of the treatment period (Days 17, 18, and 19: post-treatment). Calorimeters at both sites were previously cross validated, and the same calorimeter was used for each participant at baseline and post-treatment [28]. Participants followed the activity schedule shown in Supporting Information Table S6. Descriptions of all components of EE and substrate oxidation measured are provided in online Supporting Information. Twenty-four-hour urine was collected, urine volume and collection duration were determined, and a sample analyzed for nitrogen and ketone bodies.

Physical activity

Physical activity level (PAL) while in the calorimeter was calculated as follows: PAL = 24-hour EE/RMR.

Body composition

Dual-energy x-ray absorptiometry scanning (Lunar iDXA; GE Healthcare) was carried out in the fasted state according to standardized procedures (secondary end point).

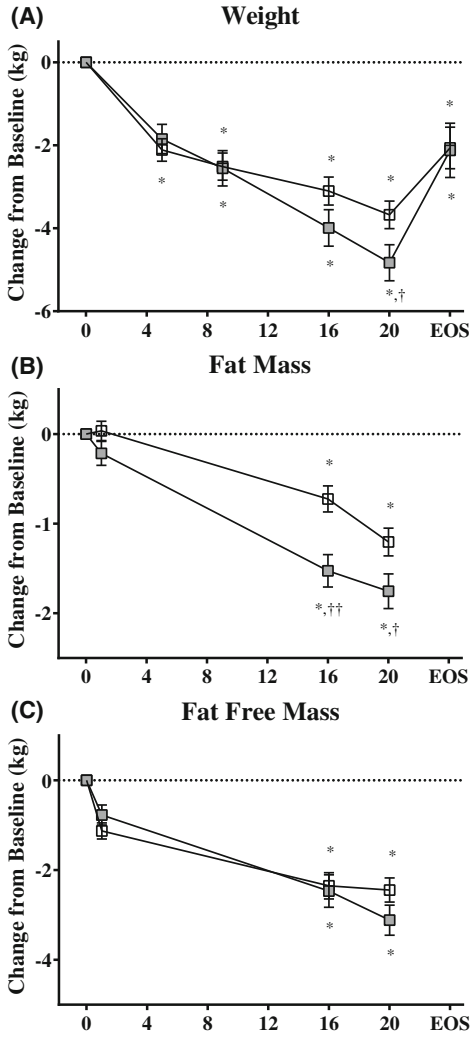

FIGURE 1 SAR425899 leads to greater weight and fat mass reductions. (A) Weight was evaluated at baseline, at Days 5, 9, 16, and 20, and at EOS (6-8 days after last dose and end of calorie-restricted diet). Body composition (by dual-energy x-ray absorptiometry) was evaluated at baseline and at Days 1, 16, and 20: (B) fat mass; (C) fat free mass. Comparisons done by repeated measures multivariate ANOVA ($n = 17$ placebo; $n = 11$ SAR425899). *Significant difference from baseline at $p < 0.05$. †Group difference at $p < 0.05$. ††Group difference at $p < 0.01$. Placebo, open box; SAR425899, gray box. EOS, end of study

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Clinical labs and biomarkers

The following pharmacodynamic circulating biomarkers were measured (secondary end points): fasting plasma glucose, hemoglobin A_{1c}, lipid biomarkers (free fatty acids, triglycerides, total cholesterol, high-density lipoprotein/low-density lipoprotein cholesterol), ketone bodies (blood and urine), and leptin.

Statistical analyses

Sample size assessment was based on the primary end point of the study to compare the change from baseline to end of treatment in SMR (kilocalories/day) between SAR425899 and placebo. The calculation was performed for a one-sided test with type I error level $\alpha = 0.05$. A value of approximately 50 kcal/d was expected for the SD for change from baseline in SMR. For power calculation, SD between 45 and 55 kcal/d and effect sizes between 36 and 84 kcal/d were used based on *in silico* trial simulations using existing validated models [24, 29, 30] using SAR425899 Phase 1 trial data as input [13]. With 12 evaluable participants per treatment arm, if the true SD was as much as 50 kcal/d and the effect size 60 kcal/d, the power was expected to be 88.5%.

Multivariate ANOVA of repeated measures was used to compare differences between calorimetry days at baseline with a group interaction factor for EE components, substrate oxidation rates including RER variables, and EI. The between-day coefficient of variation (CV) was calculated for each individual variable: $CV = \sqrt{\sum_1^N \left(\left(\sum_1^K M^2 \right) - \left(\left(\sum_1^K M \right)^2 / K \right) \right) / N(K-1)}$ (N = number of participants; K = number of days measured [3]; M = measured value each day) and relative CV (%CV, [CV/mean]) [31].

The primary analysis of SMR change from baseline was based on a linear mixed model with treatment and stratification factors (site and gender) as class effects, SMR at baseline and change in body weight as covariates, participant as a random effect, and study day as a repeating factor. Treatment difference for change from baseline was based on corresponding contrasts and

TABLE 2 Circulating metabolic biomarkers at baseline and post-intervention

	Placebo ($n = 17$)		SAR425898 ($n = 11$)		*p* value	
Variable	BASELINE, mean (SD)	POST, mean (SD)	BASELINE, mean (SD)	POST, mean (SD)	Time	Time × group
HbA_{1c} (%)	5.48 (0.29)	5.39 (0.28)	5.24 (0.50)	5.07 (0.35)	**0.004**	0.357
Glucose (mmol/L)	5.26 (0.50)	5.14 (0.35)	5.13 (0.44)	4.79 (0.37)	**0.012**	0.222
Total cholesterol (mmol/L)	4.99 (1.10)	4.48 (1.09)	4.76 (0.84)	4.07 (0.99)	**<0.0001**	0.397
HDL (mmol/L)	1.17 (0.31)	1.08 (0.28)	1.25 (0.31)	1.04 (0.21)	**<0.0001**	0.074
LDL[a] (mmol/L)	57.7 (65.4)	53.9 (62.4)	65.4 (56.6)	61.2 (57.4)	0.115	0.929
Triglycerides (mmol/L)	1.78 (0.92)	1.27 (0.64)	1.95 (2.00)	1.03 (0.75)	**0.0002**	0.232
Free fatty acids[b] (mmol/L)	0.439 (0.127)	0.464 (0.160)	0.398 (0.164)	0.612 (0.161)	**0.005**	**0.020**
Leptin (μg/L)	44.7 (35.9)	31.4 (25.5)	46.0 (29.3)	28.3 (17.2)	**<0.0001**	0.495

Note: Variables are shown for baseline (Day 1) and post-treatment (Day 20; POST). Time and time × group comparisons done by repeated measures multivariate ANOVA.
Abbreviations: HbA_{1c}, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
[a] n (placebo) = 17 and n (SAR425899) = 10.
[b] n (placebo) = 16 and n (SAR425899) = 9.

TABLE 3 Evaluation of factors related to changes in body energy stores

EI variable	Placebo	SAR425899	Difference	*p* value
EI intervention (kcal/19 days)	29,197 ± 7198	23,609 ± 8408	5588 ± 2974	0.09
Change in energy stores (kcal)	−13,927 ± 5884	−19,678 ± 6568	5752 ± 2382	0.03
EE balance (kcal)	43,123 ± 2145	43,287 ± 2666	−164 ± 3422	0.96

Note: EI intervention is the total energy intake over the 19-day study period. The change in energy stores represents the change of the body energy storage pools (in kilograms) based on measured fat mass and fat free mass (9300 [kcal/kg] × fat mass change [kg]) − (1100 [kcal/kg] × fat free mass change [kg]). EE balance represents the difference between EI and change in energy stores (EI − change in energy stores). Data are shown as mean ± SD. Comparison between placebo and SAR425899 done by an independent sample t test.
Abbreviations: EE, energy expenditure; EI, energy intake.

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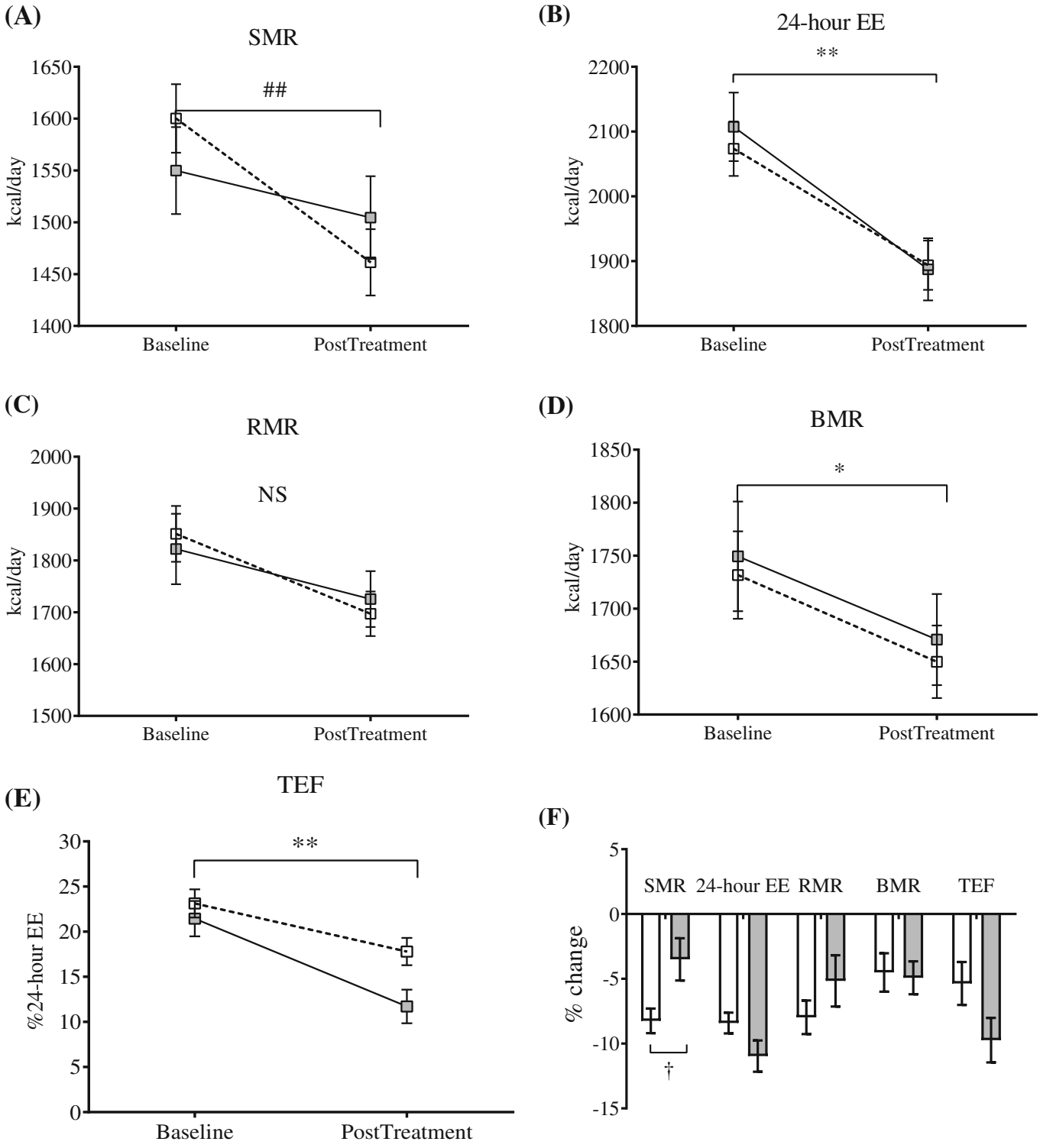

FIGURE 2 Baseline and post-intervention values and relative changes in EE components adjusted for body composition. (A-E) SMR, 24-hour EE, RMR, BMR, and TEF, respectively, at baseline and post-treatment. The reduction in SMR was lower in the SAR425899 group. (F) Percentage changes in the same components. There was smaller percentage change in SMR in the SAR425899 group. Gray and white squares and bars represent means and SEM for the SAR425899 and placebo groups, respectively. Comparisons of least-square mean absolute change done by repeated measures multivariate ANOVA and percentage change by independent sample *t* tests. $^{\#\#}p < 0.01$, for time × group effect. $^{*}p < 0.05$, $^{**}p < 0.01$, for time effect in both groups. $^{\dagger}p < 0.05$, for group effect. Placebo, open box/bar; SAR425899, gray box/bar. BMR, basal metabolic rate; EE, energy expenditure; RMR, resting metabolic rate; SMR, sleeping metabolic rate; TEF thermic effect of feeding

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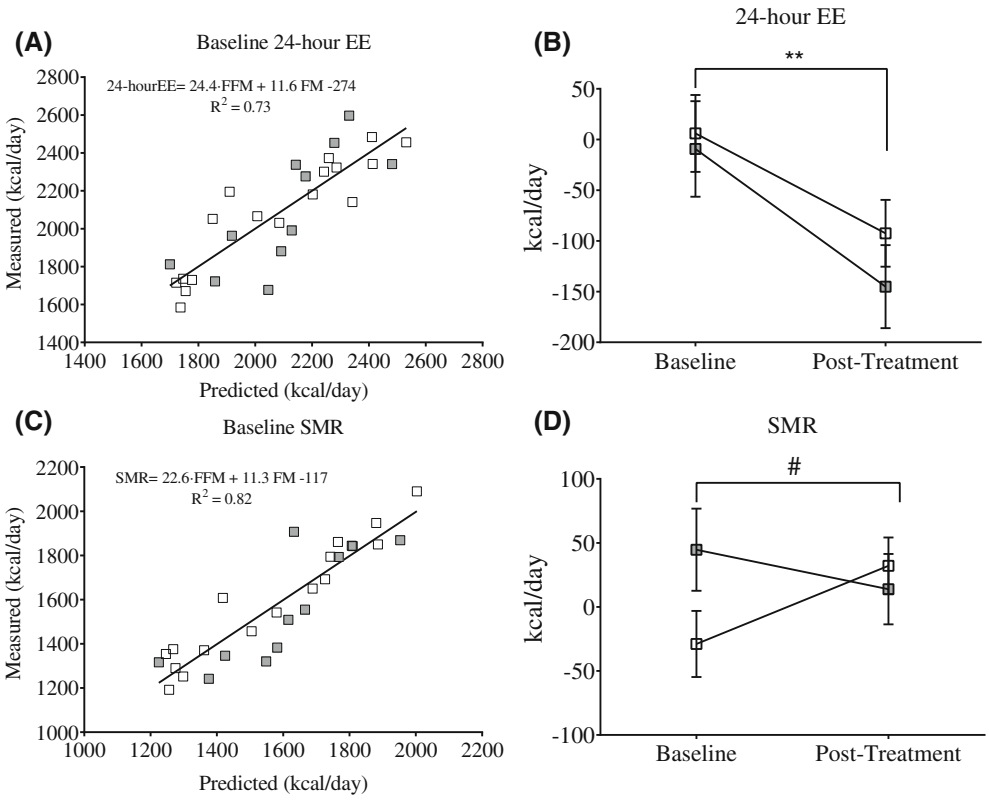

FIGURE 3 Sleeping and 24-hour metabolic adaptation. Scatterplots are measured vs. predicted (A) 24-hour EE and (C) SMR at baseline to generate the regression equations used to estimate 24-hour EE and SMR at baseline and post-treatment (average of Days 17-19). (B,D) Metabolic adaptation for 24-hour EE and SMR was calculated by the following: ([Estimated $EE_{post\text{-}treatment}$ − Estimated $EE_{baseline}$] − [Measured $EE_{post\text{-}treatment}$ − Measured $EE_{baseline}$]). $^{**}p < 0.01$, for time effect in both groups. $^{\#}p < 0.05$, for time × group effect. Placebo, open box; SAR425899, gray box. EE, energy expenditure; SMR, sleeping metabolic rate; FFM, fat free mass; FM, fat mass

reported with point estimate, two-sided 90% confidence interval (CI), and corresponding one-sided *p* value. Similar analyses were conducted for the remaining EE components and substrate oxidation variables. Changes in weight and body composition from baseline were calculated and adjusted to baseline values. Percent differences were calculated for all components of EE, and independent sample *t* tests were performed to compare differences between groups.

Metabolic adaptation was also evaluated by generating regression equations to predict EE from baseline body composition variables (FFM and FM) and used using equations to predict baseline and post-treatment 24-hour EE and SMR. Metabolic adaptation was calculated with the following equation: (Estimated $EE_{post\text{-}treatment}$ − Estimated $EE_{baseline}$) − (Measured $EE_{post\text{-}treatment}$ − Measured $EE_{baseline}$) [32].

We calculated EE balance (EEbal) using daily EI and the change of body energy storage pools (kg) via the following equation [33]:

$$EEbal = EI - \rho FFM \Delta FFM - \rho FM \Delta FM$$

where ρFFM = 1100 kcal/kg is the energy density of FFM and ρFM = 9300 kcal/kg is the energy density of body FM [34].

RESULTS

Population characteristics

The study population consisted of females and males with a mean (SD) age of 36.0 (7.7) years and a higher percentage of males enrolled in the study (67.9%). The population was diverse, with 40.7% White, 44.4% Black, 14.8% Asian, unknown, multiple, or other, and 32.1% Hispanic or Latino ethnicity. The baseline clinical characteristics indicate that we enrolled a population with overweight and obesity without evidence of diabetes or dyslipidemia, other than slightly elevated triglycerides. There were no differences in baseline characteristics between groups (Table 1).

Calorimetry data were collected over 3 days at baseline and post-treatment (Supporting Information Figure S1). Baseline EE, RER, EI, and substrate oxidation parameters are shown in Supporting Information Table S7. As expected, EE components and RER were not significantly different between groups over the three measurements at baseline (pre-treatment). At baseline, $RER_{24\text{-}h}$ reflected the provided diet (food quotient = 0.885). There was a between-day difference in $RER_{24\text{-}h}$ and a trend in basal RER ($p < 0.0001$ and 0.047, respectively), but the absolute change was quite small, had minimal variation

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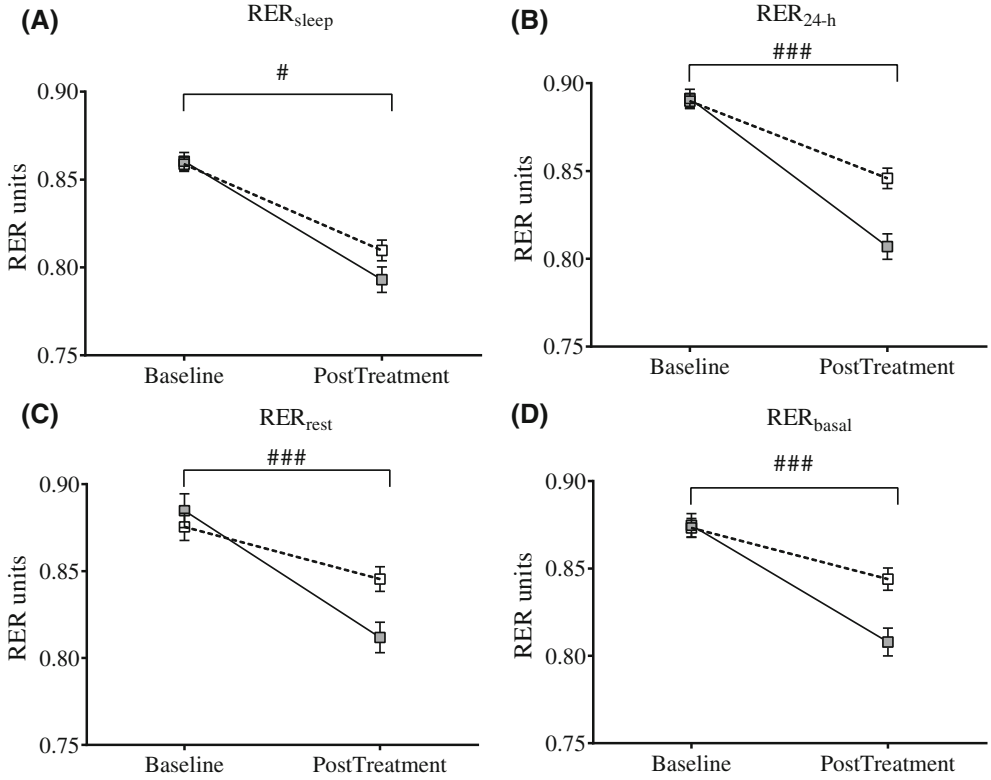

FIGURE 4 Baseline and post-intervention values for RER components. (A-D) RER_{sleep}, RER_{24-h}, RER_{rest}, and RER_{basal}, respectively, at baseline and post-intervention. The absolute reductions were significantly greater in the SAR425899 group. Gray and white squares and bars represent means and SEM for the SAR425899 and placebo groups, respectively. Comparisons of least-square mean absolute change done by repeated measures multivariate ANOVA and percent change by independent sample *t* tests. $^{\#}p < 0.05$ and $^{\#\#\#}p < 0.001$, for time × group effect. Placebo, open box; SAR425899, gray box. RER_{24-h}, 24-hour respiratory exchange ratio; RER_{basal}; basal RER; RER_{rest}, rest RER; RER_{sleep}, sleep RER

between days, remained within the expected substrate oxidation based on food quotient, and was not biologically meaningful [35]. EI, substrate oxidation (carbohydrate and fat), and urinary ketones differed slightly between days in both groups ($p = 0.008$, <0.0001, <0.0001, and <0.05, respectively), but there was no significant difference between treatment groups.

SAR425899 safety and tolerability in the safety population

No serious AEs or severe treatment emergent AEs (TEAEs) were reported during the study. TEAEs leading to permanent treatment discontinuation (Supporting Information Table S8) were reported in both the placebo group and the SAR425899 group. Gastrointestinal disorders were observed in 9/18 (50.0%) participants in the placebo group and 15/17 (88.2%) participants in the SAR425899 group. Particularly, the frequency of vomiting was high for the SAR425899 group (58.8% of all participants exposed to SAR425899), with no such events in the placebo group. The most frequently reported TEAEs, reported by ≥3 participants in either the placebo group or the SAR425899 group (for the safety population), can be found in Supporting Information Table S8 and AEs by primary organ class can be found in Supporting Information Table S9. Additional details on AE assessments are provided in online Supporting Information.

Weight loss is greater, and body composition profile is more favorable, with SAR425899 owing to a greater deficit in EI

Under conditions of calorie restriction, the mean (SD) change from baseline for body weight (adjusted for baseline weight) on Days 5, 9, 16, and 20 was −2.11 (1.11), −2.51 (1.35), −3.10 (1.39), and −3.68 (1.37) kg for placebo and −1.85 (1.17), −2.56 (1.41), −3.99 (1.46), and −4.83 (1.44) kg for SAR425899 ($p \leq 0.0001$ and $p = 0.002$ for time and interaction time × group effects, respectively; Figure 1A). The mean (SD) change from baseline for FM and FFM (kilograms) at Days 16 and 20 was −1.5 (0.5) kg and −2.4 (1.2) kg for SAR425899 and −1.0 (0.5) kg and −1.8 (0.8) kg for placebo, respectively ($p \leq 0.0001$ and $p = 0.01$ for time and interaction time × group effects, for FM; $p \leq 0.0001$ and $p = 0.02$ for time and interaction time × group effects, for FFM; Figure 1B,C). Multiple comparisons between groups and time points revealed significant between-group differences for weight and FM only (Figure 1A,B).

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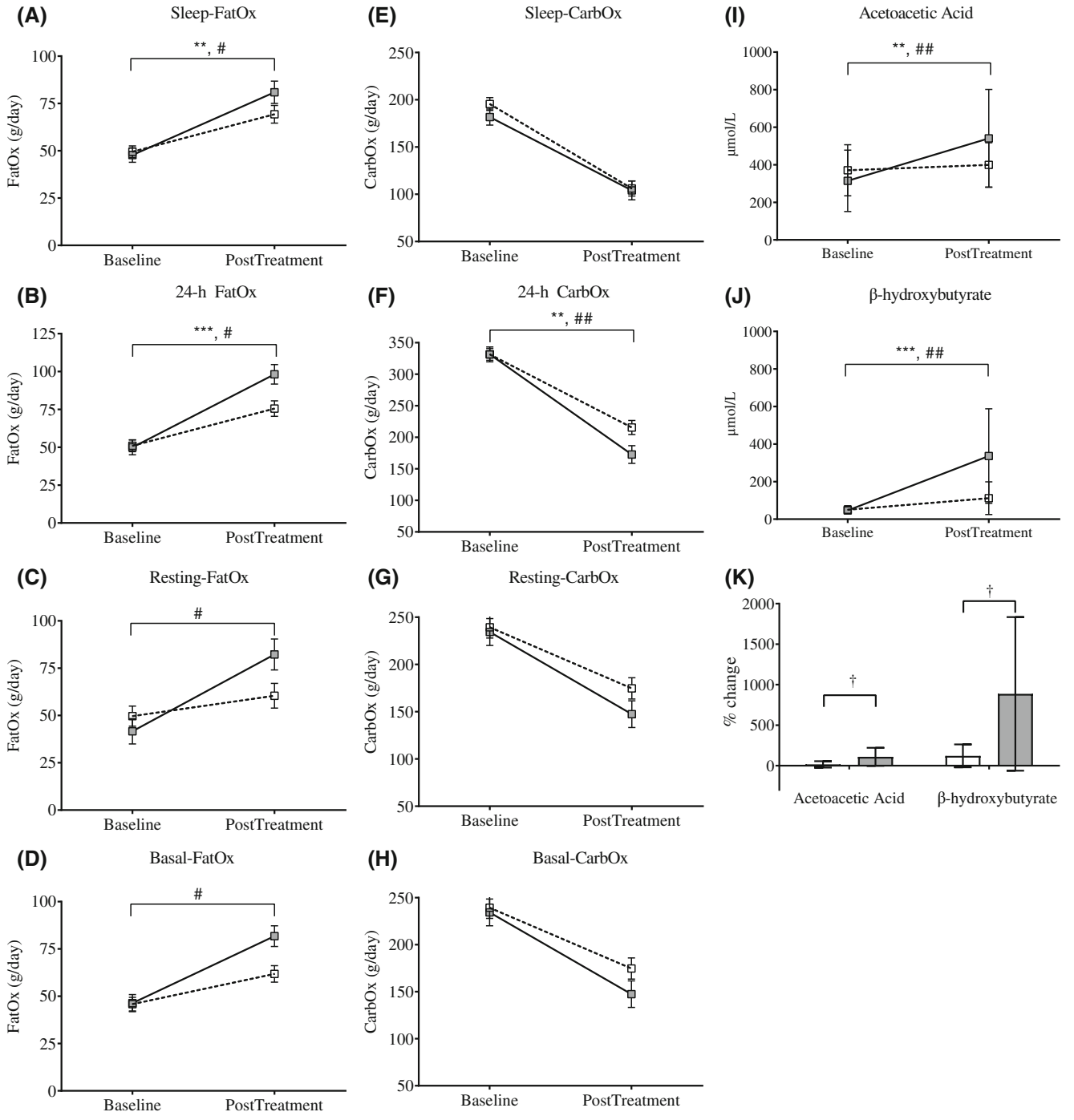

FIGURE 5 SAR425899 leads to an increase in FatOx and circulating ketone bodies. FatOx and CarbOx rates were calculated for all EE components: (A,E) sleep metabolic rate; (B,F) total daily EE (24 hours); (C,G) resting; (D,H) and basal. FatOx was higher in the SAR425899 group for sleep, 24 hours, resting, and basal. CarbOx over 24 hours was higher in the placebo group. Absolute values of (I) acetoacetic acid and (J) β-hydroxybutyrate at baseline and post-intervention. There was an increase in both groups, with a greater increase in the SAR425899 group. (K) There was also a greater percent change in the SAR425899 group in both ketones. Gray and white squares represent means and SEM for the SAR425899 and placebo groups, respectively. Comparisons of least-square mean absolute change done by repeated measures multivariate ANOVA and percent change by independent sample *t* tests. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$, for time × group effect; $^{**}p < 0.01$ and $^{***}p < 0.001$, for time effect in both groups; $^{\dagger}p < 0.05$, for group effect (panel K). Placebo, open box/bar; SAR425899, gray box/bar. CarbOx, carbohydrate oxidation; EE, energy expenditure; FatOx, fat oxidation

As expected with caloric restriction and weight loss, there were significant improvements in several metabolic parameters between Day 1 and Day 20, including glycemic control, plasma lipids, and leptin, that were similar in both groups (Table 2). Free fatty acids were increased in both groups ($p = 0.005$) with a significantly greater increase in the SAR425899 group ($p = 0.02$; Table 2).

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We next evaluated the potential impact of unintended factors on the changes in body weight and body composition. We planned an energy deficit between baseline and the treatment period of 1000 kcal/d for both groups. As expected, there was a significant daily reduction in EI from baseline, approximating 1000 kcal in each group ($p < 0.05$; Supporting Information Figure S3A). There were no statistically significant differences in EI between groups over the 19-day intervention period (Supporting Information Figure S3A; Table 3). However, there was a trend toward a higher daily change in EI in the SAR425899 group than planned (placebo −989 [489] kcal/d; SAR425899 −1442 [667] kcal/d; $p = 0.07$). We found no significant differences in PAL between placebo and SAR425899 (Supporting Information Figure S3B).

Because differences in EI were quantitatively different between groups, we evaluated the change in BES in relationship to EI by calculating EEbal [33]. This approach uses the principle of energy balance under conditions of weight loss (EI = EE + change in BES) and re-arranges the equation to solve for EE to determine whether EI was a factor in the differences in BES [36]. Similar to changes in weight and body composition, the change in BES was significantly higher in the SAR425899 group ($p = 0.03$). However, we found that EEbal was quantitatively similar between the groups ($p = 0.96$; Table 3).

Reduced metabolic adaptation with SAR425899

As expected from body weight loss, SMR declined in both treatment groups from baseline until the end of treatment ($p < 0.001$; Supporting Information Figure S4A). Although participants in the SAR425899 group lost more weight than the placebo group, the decrease from baseline for SMR (on-treatment calorimeter days, ±SD) was larger in the placebo group compared with SAR425899 ($p = 0.002$; Figure 2A) after adjusting for changes in body composition (lean mass and FM). The SMR percentage decrease from baseline for SAR4256899 adjusted for change in body composition was statistically significantly smaller than for placebo ($p = 0.001$; Figure 2F). Similar results were obtained when adjusting for baseline body weight and change in body weight (Supporting Information Figure S5A-S5B). There were significant reductions in other EE components over time (Supporting Information Figure S4B-S4D), but there were no differences between groups or after adjustment for body composition (Figure 2B–E).

To further evaluate differences in metabolic adaptation, we used regression equations to predict EE from baseline body composition variables (Figure 3A,B) and found that SAR425899 treatment led to an equivalent metabolic adaptation for 24-hour EE (Figure 3C) but a blunting of metabolic adaptation during sleep (Figure 3D).

Increased FatOx and ketogenesis with SAR425899

We found that $RER_{24\text{-}h}$ and all subcomponents decreased in both groups toward the end of treatment (Figure 4A–D). These changes were significantly greater with SAR425899 than with placebo. For example, although both groups started with an $RER_{24\text{-}h}$ of 0.89, the placebo group decreased to 0.85 [0.02] and the SAR425899 group decreased to 0.80 [0.03] ($p < 0.05$; Figure 4B).

To further define the role of substrate oxidation with SAR425899 treatment, we evaluated FatOx and carbohydrate oxidation (CarbOx) across all EE parameters. The SAR425899 group had significantly higher FatOx during sleep, total daily FatOx (24 hours), and FatOx during the basal and resting EE periods ($p = 0.01$, 0.001, 0.002, 0.003, respectively; Figure 5A–D). The only difference in CarbOx was over 24 hours, where placebo had higher CarbOx than SAR425899, likely owing to the shift to FatOx in the SAR425899 group ($p = 0.006$; Figure 5E–H).

Ketone bodies (acetoacetic acid and β hydroxybutyrate) were increased in both groups (time effect; $p < 0.01$ and <0.001 for acetoacetic acid and β hydroxybutyrate; Figure 5I,J). The increase in both ketone bodies was significantly higher in the SAR425899 group ($p < 0.01$, respectively, for interaction of time × group; Figure 5I,J). The percent change in both ketone bodies was significantly greater for SAR425899 ($p < 0.05$; Figure 5K).

The differences observed in FatOx and ketogenesis could have been due to a larger energy imbalance in the SAR425899 group. After adjusting for change in energy stores, these differences between groups remained significant (46.0 [4.8] vs. 26.0 [3.8] g/d, $p = 0.005$; 280.0 [60.8] vs. 75.4 [49.7] μmol/L, $p = 0.02$; and 226.8 [58.5] vs. 27.8 [46.1] μmol/L, $p = 0.02$ for SAR425899 vs. placebo and 24-hour FatOx, β-hydroxybutyrate, and acetoacetic acid, respectively; Supporting Information Figure S6).

DISCUSSION

To address the EE effects of dual GLP-1/GCGR agonism, we conducted a metabolic ward study under rigorous environmental controls that were intended to clamp EI. We observed a greater weight loss and reduction in FM with SAR425899 plus caloric restriction versus caloric restriction alone. This greater change in BES was at least partly due to our inability to clamp EI equivalently between groups. Despite this limitation, we found intriguing metabolic effects of SAR425899 that could be important drivers for weight loss. Specifically, we found a statistically significantly smaller reduction in SMR, but not 24-hour EE, with SAR425899 (before and after adjustment for change in body weight and body composition). The SMR results are an indication of reduced metabolic adaptation with SAR425899. We also found greater FatOx and ketogenesis that were not due to the greater changes in weight and body composition. These metabolic effects of SAR425899 are important concepts for the further development of weight-loss therapies because both low EE and low FatOx are risk factors for weight gain that impair loss of FM [12, 37].

Both preclinical and short clinical studies of the dual GLP-1R/GCGR agonist oxyntomodulin have shown increases in total, resting, and/or activity related EE [10, 38]. Neither of those studies used weight loss through calorie restriction to quantify changes in EE

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without confounding from greater metabolic adaptation in the treated group. The absolute difference in SMR over an 8-hour sleep period was ~34 kcal/d. The absolute sleep metabolic adaptation for the placebo group was 61 ± 60 kcal/d whereas in the SAR425899 group it was −31 ± 79 kcal/d. This suggests that ramping up of EE during sleep may account for some of the metabolic benefits with SAR425899 treatment. Because of the small effect on SMR, we conclude that the clinical relevance of this result remains an open question. This conclusion is supported by a study showing that SAR425899 is a weak GCGR agonist, suggesting that the GLP-1/GCGR ratio in SAR425899 may require additional optimization [39]. We found that the greater change in BES in the SAR425899 group was at least partially explained by differences in EI based on a calculation of EEbal. This implies that the observed BES differences were likely attributable to the EI differences. Therefore, our hypothesis of a role of glucagon agonism on increased EE is not entirely supported by our data, in line with our previous findings [4, 5].

Despite the limited impact of SAR425899 on EE, the blunted metabolic adaptation observed in the SAR425899 group may be important for weight-loss maintenance [12]. Our findings are supported by our recent study in which we observed reduced metabolic adaptation to be a distinguishing phenotype in people who meet their predicted weight-loss targets versus those who do not [40].

We postulated that the reduced metabolic adaptation we saw with GLP-1R/GCGR agonism under conditions of caloric restriction might be coupled with substrate switching. $RER_{24\text{-}h}$ and other subcomponents decreased in both groups toward the end of treatment, demonstrating a shift toward lipid oxidation. The change from baseline was roughly twofold higher under treatment with SAR425899 compared with placebo (22.8 vs. 50.9 g/d). This difference represents an improved negative fat energy balance of 196 g of fat per week. In line with increased flux through the lipolytic and FatOx machinery, there was an 886% increase in β-hydroxybutyrate in the SAR425899 group (vs. a 121% increase in placebo) and a 109% increase in the SAR425899 group in acetoacetic acid (vs. a 16% increase in placebo). Impaired FatOx is part of the obesity phenotype [41–43] and a key barrier to weight-loss maintenance [44, 45]. Importantly, this increased FatOx and ketogenic metabolic profile was independent of the greater changes in weight and body composition in the SAR425899 group. Therefore, our results suggest that, unlike caloric restriction [41], SAR425899 improves impairments in FatOx, and this might contribute to weight-loss success under certain conditions [12].

There were several limitations of our study. There were more dropouts in the SAR425899 group, which could have biased our results. This is unlikely given that the baseline characteristics of the people who were randomized but did not complete the study were similar to those who completed the study. We also only found metabolic adaptation effects to the study drug on SMR and not 24-hour EE. Whether this selective adaptation response is meaningful in long-term weight-loss maintenance using this drug cannot be addressed in this study. We did not have a GLP-1R arm in the study, making it difficult to know whether the observed changes are due to the presence of GCGR.

CONCLUSION

In conclusion, we uncovered effects of SAR425899 on metabolic pathways that are relevant for weight loss and weight-loss maintenance: a selective reduction in metabolic adaptation and a quantitatively relevant increase in FatOx with a parallel increase in ketogenesis. We propose that an optimal combination of GLP-1 and GCGR agonism may translate to greater weight-loss success in conditions in which EI is not clamped. Continued development of incretin-based weight-loss therapeutics would, therefore, represent a fruitful area for continued research.O

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This trial was funded by Sanofi, the study sponsor. Study drug was provided by Sanofi. Sanofi was involved in study design in collaboration with site investigators, data analysis, and manuscript review. Sanofi was not involved in data collection. The manuscript was prepared by Dr. Corbin and other members of the research team at the study sites. Sanofi was permitted to review the manuscript and suggest changes, but the final decision on content was exclusively retained by the principal investigators.

CONFLICT OF INTEREST

Steven R. Smith has received research grants or contracts from Bayer Pharma; and serves as a consultant to Novartis, Eli Lilly & Co., Novo Nordisk A/S, and Zealand Pharma. Eric Ravussin has received research grants, contracts, or unrestricted gifts from Eli Lilly & Co., ICON, Novartis, and Sanofi-Aventis; receives consulting fees from Energesis Pharmaceuticals, Eli Lilly & Co. USA, Merck, Amway, Nutrilite Health Institute-Amway, Kintai Therapeutics, YSOPIA (LNC Therapeutics), Big Sky Health, and Generian; has received payment or honoraria for lectures, presentations, speakers bureau, manuscript writing, or educational events from Open Academi-Venice; has received support for attending meetings or travel from SSIB, Grand Rounds-Oregon, Florida State University, Copenhagen Bioscience Conference, New York University, Open Academy, Aegean Conference on Precision Nutrition, Tulane Personalized Health Institute, Societe Francaise du Diabete, European Association for the Study of Obesity, and American Association of Clinical Endocrinology; and was the Editor-in-Chief of *Obesity*. Kevin D. Hall received support for the present manuscript from the NIH NIDDK. Pierre-Philippe Luyet, Britta Göbel, and Joachim Tillner are employees of Sanofi. Jaochim Tillner owns Sanofi stock. Steven R. Smith and Eric Ravussin received a research grant

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from Sanofi-Aventis, which allowed the conduct of this study. All other authors declared no conflict of interest.

CLINICAL TRIAL REGISTRATION

This trial is registered on ClinicalTrials.gov- NCT03376802.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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