Steroids 77 (2012) 323–331

Contents lists available at SciVerse ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Review

# Meal timing and composition influence ghrelin levels, appetite scores and weight loss maintenance in overweight and obese adults

Daniela Jakubowicz [a,*], Oren Froy [b], Julio Wainstein [a], Mona Boaz [c,d]

[a] Diabetes Unit, E. Wolfson Medical Center, Tel Aviv University, Holon 58100, Israel
[b] Institute of Biochemistry, Food Science and Nutrition, Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel
[c] Epidemiology and Research Unit, E. Wolfson Medical Center, Holon 58100, Israel
[d] School of Health Sciences, Department of Nutrition Sciences, Ariel University of Samaria, Israel

ARTICLE INFO

*Article history:*
Received 13 October 2011
Accepted 22 November 2011
Available online 9 December 2011

*Keywords:*
Meal timing
Diet induced weight loss
Weight regain
Craving
Ghrelin suppression

ABSTRACT

*Background:* Although dietary restriction often results in initial weight loss, the majority of obese dieters fail to maintain their reduced weight. Diet-induced weight loss results in compensatory increase of hunger, craving and decreased ghrelin suppression that encourage weight regain. A high protein and carbohydrate breakfast may overcome these compensatory changes and prevent obesity relapse.
*Methods:* In this study 193 obese (BMI 32.2 ± 1.0 kg/m$^2$), sedentary non diabetic adult men and women (47 ± 7 years) were randomized to a low carbohydrate breakfast (LCb) or an isocaloric diet with high carbohydrate and protein breakfast (HCPb). Anthropometric measures were assessed every 4 weeks. Fasting glucose, insulin, ghrelin, lipids, craving scores and breakfast meal challenge assessing hunger, satiety, insulin and ghrelin responses, were performed at baseline, after a Diet Intervention Period (Week 16) and after a Follow-up Period (Week 32).
*Results:* At Week 16, groups exhibited similar weight loss: 15.1 ± 1.9 kg in LCb group vs. 13.5 ± 2.3 kg in HCPb group, $p = 0.11$. From Week 16 to Week 32, LCb group regained 11.6 ± 2.6 kg, while the HCPb group lost additional 6.9 ± 1.7 kg. Ghrelin levels were reduced after breakfast by 45.2% and 29.5% following the HCPb and LCb, respectively. Satiety was significantly improved and hunger and craving scores significantly reduced in the HCPb group vs. the LCb group.
*Conclusion:* A high carbohydrate and protein breakfast may prevent weight regain by reducing diet-induced compensatory changes in hunger, cravings and ghrelin suppression. To achieve long-term weight loss, meal timing and macronutrient composition must counteract these compensatory mechanisms which encourage weight regain after weight loss.



**Contents**



* Corresponding author. Tel.: +972 50 810 5552 (Israel)/+1 3234107001 (USA); fax: +972 3 502 8384.
*E-mail addresses:* daniela.jak@gmail.com (D. Jakubowicz), froy@agri.huji.ac.il (O. Froy), vainstein@wolfson.health.gov.il (J. Wainstein), mboaz8@yahoo.com (M. Boaz).

0039-128X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.steroids.2011.12.006

## 1. Introduction

Weight regain after weight loss represents one of the major obstacles in the therapeutic management of overweight and obesity, undoubtedly contributing to the epidemic of overweight which now exceeds 60% in United States adults and almost 20% of children [1–5]. Although dietary restriction often results in initial weight loss, the majority of obese dieters fail to maintain their reduced weight [5]. These diets are typified by short term [3–6 months] success; however, most individuals cannot maintain such weight loss strategies over time [1,3,6–9].

Proposed predictors of weight regain after weight loss include increased subjective appetite scores, especially increased hunger and craving [6–12]. Energy and/or carbohydrate restricted weight loss diets have been shown to produce a carbohydrate withdrawal effect which further exacerbates hunger and carbohydrate cravings, ultimately resulting in weight regain [9,12–16]. The reward value of carbohydrates and the consequences of its withdrawal on hunger, cravings and satiety, are not addressed by many weight loss diets, including the more successful methods [17].

Most weight loss diets result in compensatory metabolic changes, including reduced energy expenditure [18,19], increased hunger [9,12,13,20,21] and craving scores [14–16], increased circulating ghrelin and decreased posprandial ghrelin suppression [21,22]. These alterations persist over time, even 1 year after initial weight reduction [21]; further, these changes promote weight regain after diet-induced weight loss. Long term strategies to counteract these changes and to facilitate maintenance of weight loss over time might include consideration of dietary macronutrient composition and meal timing.

Macronutrient composition of the diet has been shown to influence hunger, satiety and cravings [16,23]. Several studies have shown that dietary protein is the most satiating of the macronutrients in conditions of both energy restriction and energy balance [24–27]. It has also been shown that the addition of carbohydrates to protein leads to additional reduction of hunger and increased satiety [28–30].

Meal timing also appears to influence its satiating properties. Specifically, protein consumed at breakfast (compared to lunch or dinner) leads to greater initial and sustained feelings of fullness, increased satiety and reduced levels of the appetite-regulating hormones such as ghrelin [31–35]. Moreover, the daily addition of a carbohydrate-rich snack (i.e. sweet) to breakfast has been shown to reduce the snack's reward value decreasing cravings for sweets, breads, carbohydrates and fast food [36].

The present study was designed to address whether a change in diet macronutrient composition and meal timing impacts these metabolic outcomes (appetite and ghrelin levels) leading to long term dietary adherence and prevention of weight regain. We studied a population of overweight and obese adults and compared the effects of two isocaloric weight loss diets with different meal timing and composition on appetite, craving scores, ghrelin levels, weight loss and maintenance during two consecutive periods: (1) Diet Intervention Period; and (2) Follow-up Period.

## 2. Materials and methods

### 2.1. Study design

The present study is a randomized, treatment controlled, open clinical trial comparing the effects of two isocaloric dietary interventions with different composition and meal timing on subjective appetite scores, craving, ghrelin suppression, weight loss and maintenance.

### 2.2. Participants

The study protocol initially included 193 obese/overweight subjects (115 women), recruited from outpatient clinics by means of personal interview and advertising. Inclusion criteria were adult (age 20–65 years); overweight or obese (body-mass index 25–37 kg/m$^2$) non-diabetic [glucose <200 mg/dl 2 h after oral administration of 75 g glucose after an overnight fast]; with normal thyroid, liver and kidney function as assessed by standard blood tests. Exclusion criteria included individuals with diabetes or abnormal thyroid, liver or kidney function. Individuals who were presently dieting, using medications affecting body weight or who had experienced a change in weight >4.5 kg or a change in physical activity within the six months preceding study onset were excluded. Gastrointestinal problems possibly preventing dietary adherence; pregnancy or lactation; cancer or other characteristics [psychological or physical disabilities] deemed likely to interfere with participation in or compliance with the study were further exclusion criteria. Subjects taking antihypertensive or lipid-lowering medication were asked to maintain all medications and supplements at pre-study doses. Most subjects were sedentary at baseline and were asked to maintain their usual physical activity levels and to refrain from drinking >2 standard glasses of alcohol per week throughout the study.

The protocol and potential risks and benefits of the study were fully explained to each subject before he/she provided a written informed consent. All experimental procedures followed ethical standards of and were approved by the Institutional Review Board Helsinki Committee at the Wolfson Medical Center, Holon, Israel.

**Table 1**
Diet composition by treatment assignment and sex.

| | HCb Women | | | | LCb Women | | | |
|---|---|---|---|---|---|---|---|---|
| | Kcal | gCh (%) | gProt (%) | gFat (%) | Kcal | gCh (%) | gProt (%) | gFat (%) |
| Breakfast | 600 | 60 (40) | 45 (30) | 20 (30) | 300 | 10 (13.3) | 30 (40) | 16 (48) |
| Lunch | 500 | 10 (8) | 70 (56) | 20 (36) | 500 | 10 (8) | 70 (56) | 20 (36) |
| Dinner | 300 | 8 (10.7) | 45 (60) | 10 (30) | 600 | 16 (10.6) | 90 (60) | 20 (30) |
| Total | 1400 | 78(19.6) | 160 (48.6) | 50 (32) | 1400 | 36 (10.6) | 190 (52) | 56 (38) |
| | HCb Men | | | | LCb Men | | | |
| Breakfast | 600 | 60 (40) | 45 (30) | 20 (30) | 300 | 10 (13.3) | 30 (40) | 16 (48) |
| Lunch | 600 | 12 (8) | 84 (56) | 24 (36) | 600 | 12 (8) | 84 (56) | 24 (36) |
| Dinner | 400 | 11 (10.7) | 60 (60) | 20 (30) | 700 | 19 (10.6) | 105 (60) | 23 (30) |
| Total | 1600 | 83 (19.5) | 189 (48.7) | 64 (32) | 1600 | 41 (10.7) | 219 (52) | 63 (38) |

HCPb = high carbohydrate and protein breakfast diet. LCb = low carbohydrate breakfast diet; gCh (%) = grams of carbohydrate and %; gProt (%) = grams of protein and %; gFat (%) = grams of fat and %.

### 2.3. Diet Intervention Period (Week 0–Week 16)

Subjects were assigned to one of two isocaloric weight loss diets which differed primarily in the composition of the breakfast meal:

a) Low carbohydrate diet (LCb): a low carbohydrate diet with a low calorie, and low carbohydrate breakfast; and
b) High carbohydrate- and protein-enriched breakfast diet (HCPb) with similar composition at lunch and at dinner to the low carbohydrate diet, but with a calorie-carbohydrate-and protein-enriched breakfast. In this group, the breakfast also included a "dessert" on a daily basis. The "dessert" was a sweet food selected from the following list: chocolate, cookies, cake, ice cream, chocolate mousse or donuts.

Men were instructed to consume 1600 kcal while women were instructed to consume 1400 kcal daily. Composition of the diet interventions is presented in Table 1. In order to maintain daily energy intake constant, the dinner in the HCPb was reduced from 600 to 300 kcals for women and from 700 to 400 kcals for men (Table 1). All subjects were counseled by a registered dietitian who instructed subjects how to keep daily diet intake checklists for all foods consumed. The subjects' body weights and dietary intake checklists were monitored every 4 weeks, and dietary adjustments were made as necessary.

### 2.4. Follow-up Period (Week 16–Week 32)

At the end of the Diet Intervention Period (Week 16), both groups entered the Follow-up Period (Week 16–Week 32). Participants received individual counseling and written advice from a dietitian to continue the diets, including meal timing, followed during the Diet Intervention Period; however, they were to be self-supervised in terms of caloric restriction, and were free to eat as motivated by hunger or cravings. Nevertheless, the dietitian emphasized that the maintenance of weight loss is predicated on the participant's ability to adhere to their previously assigned weight loss strategy over time. During the Follow-up Period, subjects continued visiting the clinic every 4 weeks, with the checklist for all foods consumed, for weighing and examinations, but without dietetic counseling. Food checklists were for post-hoc analyses

### 2.5. Anthropometric measurements

Subjects were weighed every 4 weeks during the study on a Detecto Physician Beam Scale (HOSPEQ, Inc., Miami, FL), before breakfast, wearing light clothes but no shoes. Waist circumference was measured using a tape measure at the umbilicus. Blood pressure was measured with the patient in a supine position using a standard cuff and sphygmomanometer. The mean of three rested measures was recorded.

### 2.6. Fasting blood assays

All assays were performed after overnight fast on Week 0, Week 16 and Week 32, for measurement of lipids, glucose, insulin serum levels and ghrelin plasma levels.

### 2.7. Breakfast meal challenge

At three time points during the study, baseline (Week 0), Week 16 and Week 32, we conducted an acute meal challenge in which subjects consumed the breakfast prescribed by their assigned diet intervention. Specifically, subjects assigned to the HCPb diet received an enriched breakfast, as prescribed by the HCPb diet, while subjects assigned to the LCb diet received a low calorie, low carbohydrate breakfast. The breakfast meals were consumed in their entirety within 15 min. On the day of the meal challenge, each subject reported to the laboratory at 07:00 after an overnight fast. After voiding, the subject was instructed to lie in a supine position on a bed. At 07:30, a catheter was placed in an antecubital vein of the non-dominant arm and kept in the patient for the next 240 min by saline drip. Thirty minutes after the catheter was inserted, the fasting baseline blood sample was taken for measurement of insulin and ghrelin. Venous blood samples were collected before and 30, 60, 120, 180 and 240 min after breakfast to assess insulin and ghrelin responses. The appetite scores were concomitantly completed.

### 2.8. Blood analysis

Blood samples for measurement of glucose, insulin and lipid concentrations were collected in tubes with no additives and allowed to coagulate at room temperature for 30 min. Serum was isolated by centrifugation (Beckman, Fullerton, CA) at 600×*g* for 10 min at 4 °C and was frozen at −20 °C until analyzed. Serum glucose was determined by the glucose oxidase method (Beckman Glucose Analyzer, Fullerton, CA). Serum total cholesterol, HDL cholesterol, and triacylglycerols, were measured enzymatically using a Hitachi-Cobas Bio centrifugal analyzer (Roche) using standard enzymatic kits (Roche). Low-density lipoprotein cholesterol (LDL-C) was calculated according to the methods described [37]. Serum insulin was determined by a double antibody RIA [CIS Bio International, Gif-Sur Yvette-Cedex, France), Sensitivity was 2.0 μU/ml and the intra- and inter-assay variability were 4.2% and 8.8%, respectively. Homeostasis model assessment (HOMA-R) index was calculated using the following formula: fasting serum insulin [mlU/ml] × fasting serum glucose (mmol/l)/22.5 [38].

Blood samples for measurement of plasma ghrelin concentrations was collected in tubes containing EDTA and centrifuged at 3000 rpm at −4 °C for 15 min. The plasma was then separated and stored in microcentrifuge tubes at −80 °C for future analysis. Plasma total ghrelin was measured with an enzyme immunoassay kit (Phoenix Pharmaceuticals, Belmont, CA). The range of the kit was 0–261 pM/L. The assay sensitivity was 12 pM/L; the intra-assay and inter-assay coefficients of variation for the assay control was 4%. All samples from a given subject were tested in duplicate and analyzed in the same assay. Total (insulin and ghrelin) and net [visual analog scores for appetite] areas under the curve during the 4-h breakfast meal tolerance test were calculated geometrically by using the trapezoidal rule.

### 2.9. Appetite questionnaires

Appetite scores for hunger and satiety were assessed using 100-mm visual analog scales (VAS), after acute meal challenge, at the same time points blood sampling was performed. Subjects were asked to make a single vertical mark on each scale somewhere between the 0 and 100 mm extremes (e.g., not at all hungry to very hungry) to indicate their feelings at that time point. Subjects did not discuss their ratings with each other and could not refer to their previous ratings when marking the scale. Reliability and validity of using VAS for assessing measures of appetite has been reported [39].

### 2.10. Craving scores questionnaire

Food cravings were assessed using the Food Craving Inventory (FCI), a 28-item questionnaire designed to measure the frequency of overall food cravings as well as cravings for specific types of foods [40]. Cravings for specific types of foods were measured by four independent subscales, each consisting of 4–8 items within the food category: high fats [i.e., fried chicken, gravy, sausage, hot dogs, fried fish, corn bread, bacon, steaks]; sweets (i.e., cakes, cinnamon rolls, ice cream, cookies, chocolate, donuts, candy, brownies); carbohydrates/starches (i.e., sandwich bread, rice, biscuits, pasta, pancakes/waffles, rolls, cereal, baked potato]; and fast-food (i.e., pizza, French fries, hamburger, chips). Participants rated how often they experienced a craving for each of the foods using a 5-point Likert scale (1 = never, 5 = always/almost every day). In addition to the four independent subscales, an overall score was calculated by summing the subscales and represents the general food craving score. Craving scores were assessed 2 days prior to initiating the diet intervention; at Week 16 and Week 32 of the study.

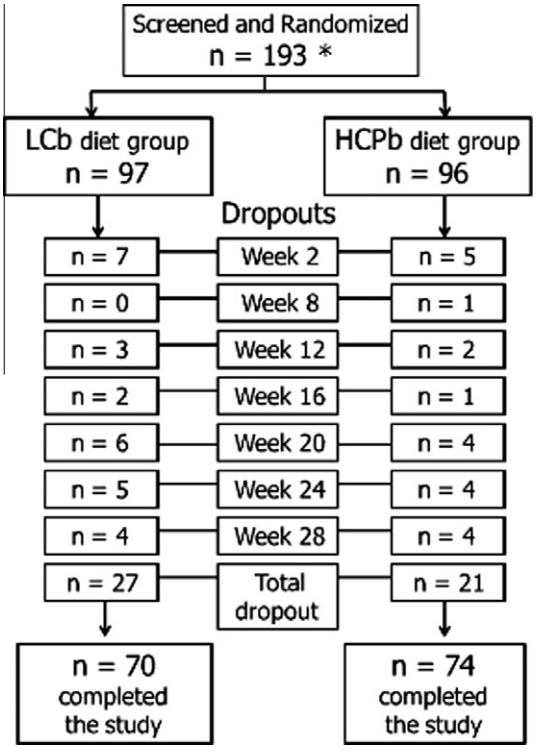


**Fig. 1.** Consort diagram. *All randomized subjects are included in the analysis per intention to-treat principle. Missing data were imputed using last observation carried forward.

### 2.11. Sample size and study power

A sample size of 130 participants (65 in each treatment group) provided 80% power to detect a true, between-group difference of 5 ± 10 kg at the end of follow-up. An additional 63 subjects were recruited to cover drop outs, which we predicted would reach almost 50% based on diet study drop-out rates in the literature.

### 2.12. Statistical analysis

All data are presented as the mean ± SEM. Statistical comparisons of group differences were performed using one-way ANOVAs combined with Tukey's post-hoc tests to compare the results between surgical groups (S-ADREC, ADREC and A-DEX) and cell treatment groups. Analysis of data was carried out using SPSS 11.0 statistical analysis software (SPSS Inc., Chicago, IL). For continuous variables, such as age, weight and biochemical measures, descriptive statistics were calculated and reported as mean ± standard deviation. Normality of distribution of continuous variables was assessed using the Kolmogorov–Smirnov test (cut off at $p = 0.01$). Normally distributed continuous variables were compared by treatment assignment using the *t*-test for independent samples, while continuous variables with distributions significantly deviating from normal were compared by treatment assignment using the Mann Whitney U. Categorical variables, such as sex and treatment assignment, were described using frequency distributions and are presented as *n* (%). A model of each of the continuous outcomes: appetite scores, cravings scores, ghrelin and body weight was developed using general linear modeling (GLM) repeated measures analyses. Treatment assignment and sex were included in all models as fixed factors and a sex-by-treatment interaction was assessed. Additionally, areas under the curve for biochemical measures, appetite and cravings scores over time were calculated using the trapezoidal rule and compared by treatment assignment using the *t*-test for independent samples. All tests follow the intention-to-treat principle and missing data were imputed using last observation carried forward. All tests are two-tailed and considered significant at $p < 0.05$.

## 3. Results

### 3.1. Patient dispensation

Of the 193 subjects (BMI=32.3 ± 1.8 kg/m$^2$) initially recruited and accepted for participation in the study, 96 (57 women and 39 men) were assigned to the HCPb group and 97 subjects (58 women and 39 men) were assigned to the LCb group. Patient dispensation is depicted in Fig. 1. As can be seen, a total of 144 participants completed the study, 74 (44 women) in HCPb group and 70 (42 women) in LCb group. Participants are compared by completion status in Table 2. In contrast to subjects who

**Table 2**
Characteristics of the study population by completion status.

| | HCPb group | | LCb group | |
|---|---|---|---|---|
| | Completed | Withdrew | Completed | Withdrew |
| | $n = 74$ | $n = 22$ | $n = 70$ | $n = 27$ |
| Follow-up time (weeks) | 32 | 16.2 ± 10.4 | 32 | 15.5 ± 10.4 |
| Age | 46.7 ± 7.1 | 42.3 ± 7.3 | 47.5 ± 6.5 | 44 ± 8.3 |
| Sex (females) | 59.5 | 59.1 | 60 | 59.3 |
| Weight week 0 (kg) | 91.2 ± 9.8 | 93.5 ± 7.5 | 90.4 ± 9.2 | 93.3 ± 7.2 |
| BMI week 0 (kg/m$^2$) | 32.2 ± 1.9 | 32.2 ± 2.0 | 32.3 ± 1.9 | 32.4 ± 1.5 |
| Weight Δ Week 0–16 (kg) | −13.6 ± 2.3 | −1.4 ± 1.6 | −15.3 ± 1.9 | −2.1 ± 2.6 |
| Hunger $AUC_{240\ min}$ | 19,391 ± 2355 | 19,343 ± 2328 | 35,628 ± 2497 | 35,374 ± 1761 |
| Satiety $AUC_{240\ min}$ | 41,460 ± 3056 | 40,882 ± 3366 | 24,966 ± 2754 | 24,936 ± 1316 |
| *Craving Scores Week 0* | | | | |
| Sweets | 12.7 ± 1.6 | 14.0 ± 2.7 | 12.3 ± 2.3 | 13.9 ± 1.8 |
| Fats | 9.7 ± 1.1 | 11.6 ± 1.1 | 9.3 ± 1.6 | 11.1 ± 2.1 |
| Carb/starches | 12.5 ± 1.5 | 12.9 ± 1.5 | 12.5 ± 1.5 | 13.0 ± 1.6 |
| Fast foods | 13.1 ± 1.5 | 12.1 ± 1.5 | 13.5 ± 1.7 | 12.7 ± 1.3 |
| General craving | 48.0 ± 4.4 | 50.5 ± 5.2 | 47.6 ± 4.9 | 50.7 ± 3.2 |

Data are indicated as mean ± SD. Compared to participants who completed the study, those who withdrew (regardless of treatment assignment) were significantly younger ($p = 0.001$); had significant higher craving scores for sweets ($p < 0.0001$), fats ($p < 0.0001$), and general craving ($p < 0.0001$), but had significant lower scores for fast food craving ($p = 0.001$). Additionally, subjects who dropped out gained weight by Week 16, while completers had lost weight at Week 16 ($p < 0.0001$).

completed the study, those who dropped out were significantly younger and had significantly higher general craving scores and craving scores for sweets and fats, and significantly lower craving scores for fast foods, regardless of treatment assignment. Additionally, subjects who withdrew had gained weight by Week 16, while those who completed the study had lost weight at this time point. Subjects who withdrew did not differ from completers in terms of sex or treatment assignment. All 193 subjects randomized to treatment are included in the analysis of results according to the intention-to-treat principle and using last observation carried forward to impute values.

### 3.2. Weight loss

At baseline, body weight was similar by treatment group (Table 3). By the end of the Diet Intervention Period (Week 16), subjects in both treatment groups lost a significant amount of weight from baseline (Fig. 2). During the Follow-up Period, from Week 16 through Week 32, subjects in the HCPb group lost additional weight, while subjects in the LCb group regained weight. Thus, at the end of the Follow-up Period (Week 32), body weight was significantly different between the two groups and was significantly lower in the HCPb than LCb group ($p < 0.0001$) (Table 3).

### 3.3. Fasting serum glucose, insulin and lipids

Fasting concentrations of glucose, insulin and HOMA-IR decreased from baseline to Week 16 in both groups. From Week 16 to Week 32, these values further declined in the HCPb group. By contrast, these values increased from Week 16 to Week 32 in the LCb group. Values differed significantly between the groups at Week 32 (Table 3). At baseline, both groups were similar in total, HDL and LDL cholesterol and triglycerides (TG). By Week 16, TG values were significantly lower and HDL values significantly higher in the LCb group. At Week 32, total cholesterol, TG and LDL were all significantly lower, while HDL was significantly higher, in the HCPb vs. LCb group (Table 3).

### 3.4. Craving scores

At baseline, none of the food craving scores differed significantly by diet intervention group. At the end of the Diet Intervention Period (Week 16), all craving scores were significantly higher in the LCb than in the HCPb group. By the end of the Follow-up Period (Week 32), all craving scores, including general cravings, sweets, high fats, carbohydrates/starches and fast foods, were significantly higher in the LCb than in the HCPb group (Table 3). The overall increase in craving scores in the LCb group was greatest for sweets, which was significantly greater than the increase in any other food category. Fat cravings were significantly greater than fast foods cravings in this group. The greatest reduction in cravings in the HCPb group was detected for sweets and fats. Other pair wise differences in cravings were not significant.

### 3.5. Cravings and weight change

Change in body weight during the Follow-up Period, Week 16 to Week 32, was significantly, positively associated with change in craving scores during the same phase. Specifically, in the Follow-up Period, weight change was associated with a change in cravings for sweets ($r = 0.24$, $p = 0.004$); carbohydrates and starches ($r = 0.2$, $p = 0.02$); fast foods ($r = 0.25$, $p = 0.003$); and general craving ($r = 0.22$, $p = 0.007$). An association between change in fats craving and change in body weight was not detected.

### 3.6. Breakfast meal challenge

#### 3.6.1. Insulin response

Insulin area under the curve [AUC] response to breakfast meal challenge did not differ between diet intervention groups at the baseline. At Week 16, both groups exhibited a significant reduction of insulin-AUC from baseline. The HCPb group exhibited a further decrease at the end of Follow-up Period, while insulin AUC significantly increased in LCb group (Table 3). As shown in Table 3, at the Week 32 breakfast meal challenge, for insulin AUC was significantly, positively associated with body weight ($r = 0.61$, $p < 0.0001$).

#### 3.6.2. Ghrelin response

The nadir ghrelin value at baseline of the breakfast meal challenge was 301.2 ± 36.0 pg/ml in the HCPb group compared to 350.2 ± 26.4 pg/ml in the LCb group ($p < 0.0001$) (Table 3). Nadir ghrelin in response to HCPb breakfast was significantly decreased from baseline to Week 16 ($p < 0.0001$) and remained suppressed at Week 32 (Fig. 3). By contrast, in the LCb group, nadir ghrelin levels did not differ significantly between baseline and Week 16 ($p = 0.06$) and were significantly less decreased after the Follow-up

**Table 3**
Participant characteristic at baseline and after 16 and 32 weeks, $n = 193$ LCb group: $n = 97$; HCPb group: $n = 96$.

| | Group | Baseline | Week 16 | Week 32 |
|---|---|---|---|---|
| Weight (kg) | HCPb | 91.2 ± 9.8 | 77.6 ± 9.0 | 70.6 ± 8.7 |
| | LCb | 90.4 ± 9.2 | 75.2 ± 8.1 | 86.9 ± 9.7 |
| | *p*-value | 0.65 | 0.11 | <0.001 |
| BMI (kg/m$^2$) | HCPb | 32.2 ± 1.9 | 27.4 ± 1.8 | 24.9 ± 1.9 |
| | LCb | 32.3 ± 1.9 | 26.9 ± 1.7 | 30.9 ± 2.0 |
| | *p*-value | 0.79 | 0.08 | <0.001 |
| Waist circumference (cm) | HCPb | 110.7 ± 3.1 | 103.3 ± 4.3 | 96.4 ± 5.3 |
| | LCb | 110.4 ± 3.2 | 102.5 ± 4.3 | 108.7 ± 3.6 |
| | *p*-value | 0.46 | 0.28 | <0.001 |
| *FASTING VALUES* | | | | |
| Fasting glucose (mg/dl) | HCPb | 94.4 ± 7.0 | 86.2 ± 5.6 | 84.2 ± 4.6 |
| | LCb | 94.6 ± 7.4 | 85.1 ± 6.7 | 95.5 ± 4.9 |
| | *p*-value | 0.81 | 0.26 | <0.001 |
| Fasting insulin (μU/ml) | HCPb | 21.7 ± 3.6 | 12 6 ± 3.4 | 8.9 ± 3.9 |
| | LCb | 21.7 ± 3.6 | 13.9 ± 4.8 | 23.69 ± 3.8 |
| | *p*-value | 0.97 | 0.30 | <0.001 |
| HOMA-IR | HCPb | 5.0 ± 0.9 | 2.5 ± 0.5 | 1.6 ± 0.4 |
| | LCb | 5.1 ± 0.9 | 2.4 ± 0.5 | 5.9 ± 0.9 |
| | *p*-value | 0.89 | 0.19 | <0.001 |
| Total cholesterol (mg/dl) | HCPb | 211.8 ± 17.6 | 189.1 ± 10.6 | 179.2 ± 11.1 |
| | LCb | 212.3 ± 19.8 | 188.6 ± 13.2 | 190.8 ± 18.2 |
| | *p*-value | 0.87 | 0.81 | <0.001 |
| Triacylglycerol (mg/dl) | HCPb | 174.4 ± 17.6 | 140.8 ± 10.9 | 122.6 ± 9.7 |
| | LCb | 174.5 ± 22.6 | 134.9 ± 7.9 | 174.5 ± 20.9 |
| | *p*-value | 0.98 | <0.001 | <0.001 |
| HDL cholesterol (mg/dl) | HCPb | 45.8 ± 5.3 | 48.8 ± 4.9 | 50.9 ± 4.9 |
| | LCb | 47.4 ± 5.3 | 51.2 ± 5.0 | 48.0.2 ± 5.0 |
| | *p*-value | N/A | N/A | N/A |
| LDL cholesterol (mg/dl) | HCPb | 157.2 ± 17.4 | 133.3 ± 10.8 | 122.2 ± 12.3 |
| | LCb | 156.2 ± 20.6 | 130.7 ± 14.2 | 134.1 ± 19.5 |
| | *p*-value | N/A | N/A | N/A |
| *CRAVING SCORES* | | | | |
| Sweets | HCPb | 12.9 ± 1.9 | 9.7 ± 3.7 | 8.4 ± 4.3 |
| | LCb | 12.78 ± 2.3 | 15.4 ± 1.8 | 17.1 ± 1.8 |
| | *p*-value | 0.34 | <0.001 | <0.001 |
| Fats | HCPb | 10.1 ± 1.8 | 9.2 ± 2.6 | 8.1 ± 2.9 |
| | LCb | 9.8 ± 1.9 | 11.3 ± 1.7 | 12.3 ± 1.9 |
| | *p*-value | 0.14 | <0.001 | <0.001 |
| Carb/starch | HCPb | 12.6 ± 1.5 | 8.8 ± 3.8 | 8.2 ± 4.1 |
| | LCb | 12.6 ± 1.6 | 15.7 ± 1.9 | 16.6 ± 1.9 |
| | *p*-value | 0.85 | <0.001 | <0.001 |
| Fast foods | HCPb | 12.8. ± 1.6 | 9.2 ± 3.6 | 8.5 ± 3.9 |
| | LCb | 13.2 ± 1.6 | 15.9 ± 1.9 | 16.6 ± 2.0 |
| | *p*-value | 0.15 | <0.001 | <0.001 |
| General craving | HCPb | 48.6 ± 4.7 | 37.1 ± 12.9 | 33.2 ± 14.7 |
| | LCb | 48.5 ± 4.8 | 58.4 ± 5.7 | 62.7 ± 6.1 |
| | *p*-value | 0.57 | <0.001 | <0.001 |
| *BREAKFAST MEAL CHALLENGE AUC* | | | | |
| Ghrelin $AUC_{240\ min}$ pg/ml × 240 min | HCPb | 219,431 ± 7479 | 204,325 ± 5579 | 201,115 ± 7295 |
| | LCb | 275,432 ± 13,873 | 280,100 ± 11,735 | 282,968 ± 9526 |
| | *p*-value | <0.001 | <0.001 | <0.001 |
| Ghrelin nadir (pg/ml) | HCPb | 300.7 ± 35.9 | 243.3 ± 13.6 | 239.1 ± 23.4 |
| | LCb | 350.5 ± 26.6 | 357.6 ± 17.1 | 363.9 ± 20.5 |
| | *p*-value | <0.001 | <0.001 | <0.001 |
| Insulin $AUC_{240\ min}$ μU/ml × 240 min | HCPb | 28,564 ± 3543 | 20,282 ± 3031 | 14,798 ± 4364 |
| | LCb | 29,066 ± 3001 | 18,050 ± 3859 | 29,816 ± 5863 |
| | *p*-value | 0.34 | <0.001 | <0.001 |
| Hunger $AUC_{240\ min}$ | HCPb | 19,346 ± 2310 | 19,301 ± 2475 | 19,890 ± 2204 |
| | LCb | 35,499 ± 2436 | 40,651 ± 3264 | 40,639 ± 3110 |
| | *p*-value | <0.001 | <0.001 | <0.001 |
| Satiety $AUC_{240\ min}$ | HCPb | 41,407 ± 3035 | 41,047 ± 3683 | 41,749 ± 2872 |
| | LCb | 24,955 ± 2736 | 26,200 ± 6852 | 25,320 ± 2844 |
| | *p*-value | <0.001 | <0.001 | <0.001 |

Data are indicated as mean ± SD. HCPb = energy-, carbohydrate- and protein-enriched breakfast diet; LCb = low carbohydrate breakfast diet. Conversion factors (metric units to SI units); glucose, mg/dl × 0.056 = mmol/l; insulin, μU/ml × 6.0 = pmol/L; ghrelin, pg/ml × 3.371 = pmol/L; total cholesterol, mg/dl × 0.0259 = mmol/l; triacylglycerol, mg/dl × 0.0113 = mmol/l; HDL-cholesterol, mg/dl × 0.0259 = mmol/l.

Period, ($p = 0.03$) in the LCb group. In the HCPb group after the Follow-up Period at Week 32, nadir ghrelin levels were significantly lower than at the end of the Follow-up Period in the LCb group ($p < 0.0001$) (Table 3). Nadir ghrelin, was significantly, inversely correlated with body weight after Diet Intervention Period ($r = -0.35$, $p < 0.0001$) and after Follow-up Period ($r = -0.42$, $p < 0.0001$) in both groups. Additionally, nadir ghrelin was positively correlated with all cravings scores at Week 16 and Week 32.

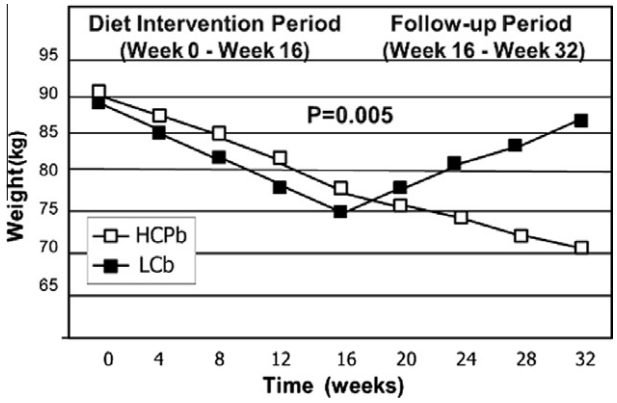


**Fig. 2.** Body weight by Diet Intervention Group. The *p*-value is for general linear model repeated measures comparisons. HCPb = energy-, carbohydrate- and protein-enriched breakfast diet group, white squares: □ LCb = low carbohydrate breakfast diet group, black squares: ■.

*3.6.3. Hunger, satiety VAS scores*

At each breakfast challenge: baseline, Week 16 and Week 32, hunger AUC was significantly lower, while satiety AUC was significantly higher after the breakfast in the HCPb group than in LCb group ($p < 0.0001$) (Table 3). In the HCPb group, significant differences in satiety and hunger scores were not detected from challenge to challenge. By contrast, a significant increase in hunger was observed in the LCb group between baseline and after the Follow-up Period.

## 4. Discussion

In this study we observed that two isocaloric diets which differed in meal timing and composition resulted in similar weight reduction at the end of the Diet Intervention Period. Weight regain after diet-induced weight loss was observed only in the LCb group, as has been reported in previous studies [4]. Subjects in the HCPb group were more successful in maintaining reduced weight; moreover, they continued losing weight during the Follow-up Period. Possible explanatory mechanisms for this between-group difference in weight maintenance outcomes include the different influence of both of the assigned diets on appetite, cravings and posprandial ghrelin levels.

Hunger and satiety response after the breakfast meal at baseline were consistent with previous reports [30,31,34]. Specifically, hunger scores were significantly lower and satiety scores significantly higher in the HCPb compared to the LCb group. By the end

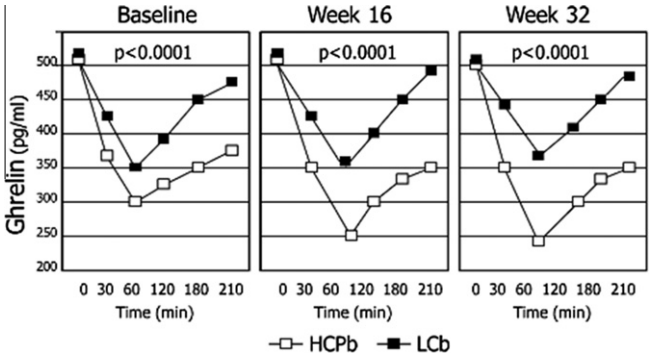


**Fig. 3.** Ghrelin suppression after breakfast meal challenge at baseline, Week 16 and Week 32 by diet intervention group. The *p*-values are for GLM repeated measures comparison by group. HCPb = energy-, carbohydrate- and protein-enriched breakfast diet group, white squares: □ LCb = low carbohydrate breakfast diet group, black squares: ■.

of Diet Intervention Period, despite similar weight reduction in both groups, hunger scores increased significantly in the LCb group. This group reported significantly more hunger than subjects in the HCPb group. Contrastly, weight reduction was not associated with an increase in posprandial hunger in the HCPb group; furthermore, HCPb subjects continued losing weight during the Follow-up Period and continued to report suppressed hunger throughout this period. This effect of an enriched breakfast on hunger and satiety persisted over time and was not less pronounced at Week 32 than after the baseline breakfast meal challenge, indicating a persistence of the treatment effect even in individuals habituated to a large breakfast [30]. These findings suggest that an enriched breakfast may represent a useful strategy to maintain weight loss and prevent weight regain over time.

All craving scores decreased in the HCPb group, especially for sweets and fats. By contrast, an overall increase in craving was observed in the LCb group, including general cravings and cravings for sweets, high fats, carbohydrates/starches and fast foods. The greatest between-group difference was craving for sweets, which were significantly higher in the LCb than in the HCPb group. Increased craving, particularly craving for sweets, was strongly associated with the regain of weight observed during the Follow-up Period in the LCb group. The weight reduction observed in the HCPb group during the Follow-up Period was correlated with decreased craving scores, especially for sweets and fats.

In many weight loss diets, energy is restricted concomitantly with the restricted intake of preferred foods, leading to an increase in the reinforcement value of the omitted or restricted food. This may be expressed as increased cravings for the desired food [14,41]. In contrast, repeated reinforcer presentation leads to a reduction of reinforcer efficacy and reduced motivation to obtain the desired food [36,42]. It is possible that the consumption of sweets at breakfast in the HCPb diet group [chocolate bar, chocolate mousse, cake, or donut] represents repeated reinforcement leading to reduced cravings.

Ghrelin suppression has been shown to be impaired in obese subjects, suggesting a defect in ghrelin-induced satiety mechanisms [43]. In this study, even before weight reduction, ghrelin levels were significantly more suppressed after HCPb than LCb breakfast, suggesting that breakfast composition might overcome the obesity related defect in ghrelin suppression. This between-group difference in ghrelin suppression is also consistent with previous reports showing greater ghrelin suppression after carbohydrate enriched vs. protein- or lipid-enriched meals [44,45].

Recent studies have shown that diet induced weight loss is associated with decreased posprandial ghrelin suppression, that persist over long time and that would be expected to facilitate regain of lost weight [21]. Despite similar weight loss in both groups at the end of the Diet Intervention Period, the association between-diet induced weight loss and decreased posprandial ghrelin suppression was seen only in the LCb group. By contrast, HCPb group subjects exhibited a significant increase in ghrelin suppression at Week 16. This suggests an improvement of ghrelin suppression after diet-induced weight loss which occurs selectively following a carbohydrate-enriched breakfast [46]. Moreover, despite additional weight loss in the Follow-up Period in the HCPb group, nadir posprandial ghrelin remained suppressed. This implies that in the HCPb group, meal timing or diet composition or both, overcame or prevented the decrease of ghrelin suppression as has been shown in previous studies [21,22].

Cravings, especially for sweets and carbohydrates/starches, have been shown to be associated with ghrelin levels [47]. The strong association between nadir ghrelin levels and all craving scores categories observed in our study may represent an alternative mechanism through which in the HCPb group the craving scores were significantly reduced.

Findings of the present study must be considered in the framework of the study's limitations. First, the between-group similarity in weight loss at Week 16 suggests similar within-group compliance, and the large between-group weight difference at Week 32 suggests that LCb subjects ceased dietary compliance while the subjects in the HCPb group maintained adherence even in the Follow-up Period. On the other hand, subjects in the HCPb group consumed added protein and carbohydrates in the morning, while the LCb group consumed a higher energy meal in the evening. This was necessary to ensure that the two diets remained isocaloric. Subjects in both groups lost weight until Week 16, indicating that both calorie-restricted diets resulted but in short term weight loss. The direct effects of meal timing (morning vs. evening consumption of carbohydrates) were not tested; however, this is the subject of our ongoing study.

In summary, increased hunger and craving scores coupled with decreased ghrelin suppression after diet induced weight loss in the LCb group was correlated with failed maintenance of weight reduction; on the contrary, progressive weight regain was observed during the Follow-up Period. This suggests that LCb subjects were not able to comply with this weight loss strategy over time. Subjects in the HCPb group continued losing weight during the Follow-up Period, implying that a carbohydrate- and protein-enriched diet may represent a strategy with which individuals can comply over the long term.

## 5. Conclusion

We found that the compensatory changes of appetite, craving and circulating as well as posprandial ghrelin that facilitate obesity relapse after diet-induced weight loss was prevented by addition of high carbohydrate, protein and calorie enriched breakfast. To achieve long term weight loss, the diet meal timing and macronutrient composition has to counteract the compensatory mechanisms that encourage weight regain after weight loss.

## References


[1] Maclean PS, Bergouignan A, Cornier MA, Jackman MR. Biology's response to dieting: the impetus for weight regain. Am J Physiol Regul Integr Comp Physiol 2011;301:R581–600.
[2] Kraschnewski JL, Boan J, Esposito J, Sherwood NE, Lehman EB, Kephart DK, Sciamanna CN. Long-term weight loss maintenance in the United States. Int J Obes (Lond) 2010;34:1644–54.
[3] Weiss EC, Galuska DA, Kettel Khan L, Gillespie C, Serdula MK. Weight regain in U.S. adults who experienced substantial weight loss, 1999–2002. Am J Prev Med 2007;33:34–40.
[4] Anderson JW, Konz EC, Frederich RC, Wood CL. Long-term weight-loss maintenance. a meta-analysis of US studies. Am J Clin Nutr 2001;74:579–84.
[5] Wing RR, Hill JO. Successful weight loss maintenance. Annu Rev Nutr 2001;21:323–41.
[6] Elfhag K, Rossner S. Who succeeds in maintaining weight loss? A conceptual review of factors associated with weight loss maintenance and weight regain. Obes Rev 2005;6:67–85.
[7] Phelan S, Wing RR, Loria CM, Kim Y, Lewis CE. Prevalence and predictors of weight loss maintenance in a biracial cohort: results from the coronary artery risk development in young adults study. Am J Prev Med 2010;39:546–54.
[8] Blundell JE, Finlayson G. Is susceptibility to weight gain characterized by homeostatic or hedonic risk factors for overconsumption? Physiol Behav 2004;82:21–5.
[9] Markowitz JT, Butryn ML, Lowe MR. Perceived deprivation, restrained eating and susceptibility to weight gain. Appetite 2008;51(3):720–2.
[10] McGuire MT, Wing RR, Klem ML, Lang W, Hill JO. What predicts weight regain in a group of successful weight losers? J Consult Clin Psychol 1999;67:177–85.
[11] Vogels N, Westerterp-Plantenga MS. Successful long-term weight maintenance. a 2-year follow-up. Obesity (Silver Spring) 2007;15:1258–66.
[12] Gilbert JA, Drapeau V, Astrup A, Tremblay A. Relationship between diet-induced changes in body fat and appetite sensations in women. Appetite 2009;52:809–12.
[13] Doucet E, St-Pierre S, Alméras N, Tremblay A. Relation between appetite ratings before and after a standard meal and estimates of daily energy intake in obese and reduced obese individuals. Appetite 2003;40:137–43.
[14] Gilhooly CH, Das SK, Golden JK, McCrory MA, Dallal GE, Saltzman E, Kramer FM, Roberts SB. Food cravings and energy regulation: the characteristics of craved foods and their relationship with eating behaviors and weight change during 6 months of dietary energy restriction. Int J Obes (Lond) 2007;31:1849–58.
[15] Coelho JS, Polivy J, Herman CP. Selective carbohydrate or protein restriction: Effects on subsequent food intake and cravings. Appetite 2006;47:352–60.
[16] Epstein LH, Carr KA, Lin H, Fletcher KD. Food reinforcement, energy intake, and macronutrient choice. Am J Clin Nutr 2011;94:12–8.
[17] Dansinger ML, Gleason JA, Griffith JL, Selker HP, Schaefer EJ. Comparison of the Atkins, Ornish, Weight Watchers, and Zone diets for weight loss and heart disease risk reduction: a randomized trial. Jama 2005;293:43–53.
[18] Leibel RL, Hirsch J. Diminished energy requirements in reduced-obese patients. Metabolism 1984;33:164–70.
[19] Rosenbaum M, Hirsch J, Gallagher DA, Leibel RL. Long-term persistence of adaptive thermogenesis in subjects who have maintained a reduced body weight. Am J Clin Nutr 2008;88:906–12.
[20] Keim NL, Stern JS, Havel PJ. Relation between circulating leptin concentrations and appetite during a prolonged, moderate energy deficit in women. Am J Clin Nutr 1998;68:794–801.
[21] Sumithran P, Prendergast LA, Delbridge E, Purcell K, Shulkes A, Kriketos A, Proietto J. Long-term persistence of hormonal adaptations to weight loss. N Engl J Med. 2011;365:1597–604.
[22] Cummings DE, Weigle DS, Frayo S, et al. Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. N Engl J Med 2002;346:1623–30.
[23] Dye L, Blundell J. Functional foods: psychological and behavioural functions. Br J Nutr 2002;88(Suppl 2):S187–211.
[24] Halton TL, Hu FB. The effects of high protein diets on thermogenesis, satiety and weight loss: a critical review. Am Coll Nutr 2004;23(5):373–85.
[25] Westerterp-Plantenga MS, Nieuwenhuizen A, Tomé D, Soenen S, Westerterp KR. Dietary protein, weight loss, and weight maintenance. Annu Rev Nutr. 2009;29:21–41.
[26] Veldhorst M, Smeets A, Soenen S, Hochstenbach-Waelen A, Hursel R, Diepvens K, Lejeune M, Luscombe-Marsh N, Westerterp-Plantenga M. Protein-induced satiety: effects and mechanisms of different proteins. Physiol Behav 2008;94:300–7.
[27] Lejeune M, Kovacs EM, Westerterp-Plantenga MS. Additional protein intake limits weight regain after weight loss in humans. Br J Nutr 2005;93:281–9.
[28] Holt SH, Delargy HJ, Lawton CL, Blundell JE. The effects of high-carbohydrate vs high-fat breakfasts on feelings of fullness and alertness, and subsequent food intake. Int J Food Sci Nutr 1999;50(1):13–28.
[29] Isaksson H, Rakha A, Andersson R, Fredriksson H, Olsson J, Aman P. Rye kernel breakfast increases satiety in the afternoon -an effect of food structure. Nutr J 2011;10:31.
[30] Astbury NM, Taylor MA, Macdonald IA. Breakfast consumption affects appetite, energy intake, and the metabolic and endocrine responses to foods consumed later in the day in male habitual breakfast eaters. J Nutr 2011;141:1381–9.
[31] de Castro JM. The time of day and the proportions of macronutrients eaten are related to total daily food intake. Br J Nutr 2007;98:1077–83.
[32] Leidy HJ, Mattes RD, Campbell WW. Effects of acute and chronic protein intake on metabolism, appetite, and ghrelin during weight loss. Obesity (Silver Spring) 2007;15:1215–25.
[33] Leidy HJ, Bossingham MJ, Mattes RD, Campbell WW. Increased dietary protein consumed at breakfast leads to an initial and sustained feeling of fullness during energy restriction compared to other meal times. Br J Nutr 2009;101:798–803.
[34] Leidy HJ, Racki EM. The addition of a protein-rich breakfast and its effects on acute appetite control and food intake in 'breakfast-skipping' adolescents. Int J Obes (Lond) 2010;34:1125–33.
[35] Jakubowicz D, Maman D, Essah P. Effect of diet with high carbohydrate and protein breakfast on weight loss and appetite in obese women with metabolic syndrome. Endocrine News 2008; Suppl I: 12.
[36] Temple JL, Chappel A, Shalik J, Volcy S, Epstein LH. Daily consumption of individual snack foods decreases their reinforcing value. Eat Behav 2008;9:267–76.
[37] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.
[38] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
[39] Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. Int J Obes Relat Metab Disord 2000;24:38–48.
[40] White MA, Whisenhunt BL, Williamson DA, Greenway FL, Netemeyer RG. Development and validation of the food-craving inventory. Obes Res 2002;10:107–14.
[41] Epstein LH, Truesdale R, Wojcik A, Paluch RA, Raynor HA. Effects of deprivation on hedonics and reinforcing value of food. Physiol Behav 2003;78:221–7.
[42] Murphy ES, McSweeney FK, Smith RG, McComas JJ. Dynamic changes in reinforcer effectiveness: Theoretical, methodological, and practical implications for applied research. J Appl Behav Anal 2003;36:421–38.
[43] Erdmann J, Lippl F, Wagenpfeil S, Schusdziarra V. Differential association of basal and postprandial plasma ghrelin with leptin, insulin, and type 2 diabetes. Diabetes 2005;54:1371–8.

[44] Foster-Schubert KE, Overduin J, Prudom CE, Liu J, Callahan HS, Gaylinn BD, Thorner MO, Cummings DE. Acyl and total ghrelin are suppressed strongly by ingested proteins, weakly by lipids, and biphasically by carbohydrates. J Clin Endocrinol Metab 2008;93:1971–9.
[45] Williams DL, Cummings DE. Regulation of ghrelin in physiologic and pathophysiologic states. J Nutr 2005;135:1320–5.
[46] Romon M, Gomila S, Hincker P, Soudan B, Dallongeville J. Influence of weight loss on plasma ghrelin responses to high-fat and high-carbohydrate test meals in obese women. J Clin Endocrinol Metab 2006;91:1034–41.
[47] Landgren S, Simms JA, Thelle DS, Strandhagen E, Bartlett SE, Engel JA, Jerlhag E. The ghrelin signalling system is involved in the consumption of sweets. PLoS One 2011;6:e18170.

# Update

**Steroids**

Volume 77, Issue 8–9, July 2012, Page 887–889

DOI: https://doi.org/10.1016/j.steroids.2012.04.014

Steroids 77 (2012) 887–889

Contents lists available at SciVerse ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Corrigendum

# Corrigendum to "Meal timing and composition influence ghrelin levels, appetite scores and weight loss maintenance in overweight and obese adults" [Steroids 77 (2012) 323–331]

Daniela Jakubowicz [a,*], Oren Froy [b], Julio Wainstein [a], Mona Boaz [c,d]

[a] Diabetes Unit, E. Wolfson Medical Center, Tel Aviv University, Holon 58100, Israel
[b] Institute of Biochemistry, Food Science and Nutrition, Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel
[c] Epidemiology and Research Unit, E. Wolfson Medical Center, Holon 58100, Israel
[d] School of Health Sciences, Department of Nutrition Sciences, Ariel University Center of Samaria, Israel

The authors regret there are errors in Tables 1 and 3. Additionally, as requested by the reviewer, we calculated dietary compliance and included an additional table and a discussion on non-compliance events and its correlation with body weight, BMI and craving scores. Finally, we refer to Conflict of interest, and correct the name of the affiliation of one of the authors.

The authors would like to apologize for any inconvenience this may have caused to the readers of the journal.

We would like to replace the original Table 1 with this table using the forward calculations.

The reviewer calculated expected values by multiplying grams of macronutrients by their anticipated calorie value, summing them and dividing by the new value for total energy. By applying the same method, we arrived at the values of the present table. As can be seen, the values are quite similar, with the exception of dinner among men in the HCPb group. Due to the increase in total energy (400 originally reported, 464 using the forward calculations), the % protein declines from 60% to 52%, and the % fat increases from 30% to 39%. The% total protein and % total fat for a given day remains consistent with our original calculations.

## 1. Diet compliance

Definition of compliance was:

Non-compliance was defined as a deviation of 10% or more from the recommended energy intake. Thus, for men, if energy intake on a given day exceeded 1760 kcal, non-compliance was recorded. For women, if energy intake on a given day exceeded 1540 kcal, non-compliance was recorded. Non-compliance was recorded as events, so that a given individual could conceivably contribute approximately 120 non-compliance events in a given 3-month period.

Using our definition, non-compliance was defined as a deviation of 10% or more from the recommended energy intake. Fig. 4 illustrates the median non-compliance events during each 4-week follow-up period. As shown, non-compliance events are compared by group and, separately, by completion status. Subjects in the LCb group exhibited significantly more non-compliance overall ($p < 0.0001$), beginning at Week 8. In pair-wise comparisons, non-compliance events increased in both groups from visit to through Week 20, at which point it stabilized in both groups but remained significantly higher in the LCb than HCPb group. Non-compliance was observed significantly more frequently among drop-outs than among those who completed the full 32-weeks of follow-up ($p < 0.0001$). This divergence also became significant at Week 8, and remained significantly higher among drop-outs than completers through Week 28 (there were no drop-outs left at Week 32). A by-sex difference in compliance was not detected. Non-compliance was significantly, positively associated with both BMI and weight, beginning in Week 8. Associations between non-compliance events, weight and BMI are shown in Table 4. Also shown in Table 4 are the significant, positive associations between each of the craving scores, the general craving scores and non-compliance.

The following paragraph should be added to the Discussion:

Cravings were significantly, positively associated with non-compliance events in the study population. These events were more frequently observed in the LCb than the HCPb group, and were positively associated with BMI and body weight. This suggests that compliance was more difficult in the LCb group, and implicates non-compliance as a mechanism underlying the between-group difference in body weight at the end of the study. It is possible that the inclusion of dessert at breakfast facilitated compliance in the HCPb group, perhaps by reducing the reward value of these foods, as stated above. Ultimately, for clinical purposes, the underlying explanatory mechanism may be less critical than the observation that the HCPb diet was associated with superior compliance and long term maintenance of weight loss.

Interestingly, non-compliance was significantly more frequently observed in the LCb than the HCPb group throughout the

DOI of original article: http://dx.doi.org/10.1016/j.steroids.2011.12.006
* Corresponding author. Tel.: +972 50 810 5552/+1 3234107001; fax: +972 3 502 8384.
*E-mail addresses:* daniela.jak@gmail.com (D. Jakubowicz), froy@agri.huji.ac.il (O. Froy), vainstein@wolfson.health.gov.il (J. Wainstein), mboaz8@yahoo.com (M. Boaz).

0039-128X/$ - see front matter 
http://dx.doi.org/10.1016/j.steroids.2012.04.014

**Table 1**
Diet composition by treatment assignment and sex.

| | HCb women | | | | LCb women | | | |
|---|---|---|---|---|---|---|---|---|
| | Kcal | gCh (%) | gProt (%) | gFat (%) | Kcal | gCh (%) | gProt (%) | gFat (%) |
| Breakfast | 600 | 60 (40) | 45 (30) | 20 (30) | 304 | 10 (13) | 30 (40) | 16 (47) |
| Lunch | 500 | 10 (8) | 70 (56) | 20 (36) | 500 | 10 (8) | 70 (56) | 20 (36) |
| Dinner | 302 | 8 (11) | 45 (60) | 10 (30) | 604 | 16 (10) | 90 (60) | 20 (30) |
| Total | 1402 | 78(22) | 160 (46) | 50 (32) | 1408 | 36 (10) | 190 (54) | 56 (36) |
| | HCb Men | | | | LCb Men | | | |
| Breakfast | 600 | 60 (40) | 45 (30) | 20 (30) | 304 | 10 (13) | 30 (40) | 16 (47) |
| Lunch | 600 | 12 (8) | 84 (56) | 24 (36) | 600 | 12 (8) | 84 (56) | 24 (36) |
| Dinner | 464 | 11 (9) | 60 (52) | 20 (39) | 703 | 19 (11) | 105 (60) | 23 (29) |
| Total | 1664 | 83 (20) | 189 (45) | 64 (35) | 1607 | 41 (11) | 219 (54) | 63 (35) |

HCPb = high carbohydrate and protein breakfast diet. LCb = low carbohydrate breakfast diet; gCh (%) = grams of carbohydrate and %; gProt (%) = grams of protein and %; gFat (%) = grams of fat and %.

**Table 4**
Association with non-compliance events for the corresponding week.

| | | | |
|---|---|---|---|
| Weight | Weight week 4 | *r*-value | 0.07 |
| | | *p*-value | 0.34 |
| | Weight week 8 | *r*-value | 0.12 |
| | | *p*-value | 0.1 |
| | Weight week 12 | *r*-value | 0.32 |
| | | *p*-value | <0.001 |
| | Weight week 16 | *r*-value | 0.29 |
| | | *p*-value | <0.001 |
| | Weight week 20 | *r*-value | 0.32 |
| | | *p*-value | <0.001 |
| | Weight week 24 | *r*-value | 0.52 |
| | | *p*-value | <0.001 |
| | Weight week 28 | *r*-value | 0.51 |
| | | *p*-value | <0.001 |
| | Weight week 32 | *r*-value | 0.64 |
| | | *p*-value | <0.001 |
| BMI | BMI week 4 | *r*-value | -0.05 |
| | | *p*-value | 0.48 |
| | BMI week 8 | *r*-value | 0.22 |
| | | *p*-value | 0.003 |
| | BMI week 12 | *r*-value | 0.39 |
| | | *p*-value | <0.001 |
| | BMI week 16 | *r*-value | 0.41 |
| | | *p*-value | <0.001 |
| | BMI week 20 | *r*-value | 0.41 |
| | | *p*-value | <0.001 |
| | BMI week 24 | *r*-value | 0.61 |
| | | *p*-value | <0.001 |
| | BMI week 28 | *r*-value | 0.61 |
| | | *p*-value | <0.001 |
| | BMI week 32 | *r*-value | 0.76 |
| | | *p*-value | <0.001 |
| Craving Scores Week 16 | Sweets | *r*-value | 0.47 |
| | | *p*-value | <0.001 |
| | Fats | *r*-value | 0.28 |
| | | *p*-value | 0.001 |
| | Starches | *r*-value | 0.5 |
| | | *p*-value | <0.001 |
| | Fast Foods | *r*-value | 0.46 |
| | | *p*-value | <0.001 |
| | General | *r*-value | 0.41 |
| | | *p*-value | <0.001 |
| Craving Scores Week 32 | Sweets | *r*-value | 0.86 |
| | | *p*-value | <0.001 |
| | Fats | *r*-value | 0.89 |
| | | *p*-value | <0.001 |
| | Starches | *r*-value | 0.88 |
| | | *p*-value | <0.001 |
| | Fast Foods | *r*-value | 0.88 |
| | | *p*-value | <0.001 |
| | General | *r*-value | 0.76 |
| | | *p*-value | <0.001 |

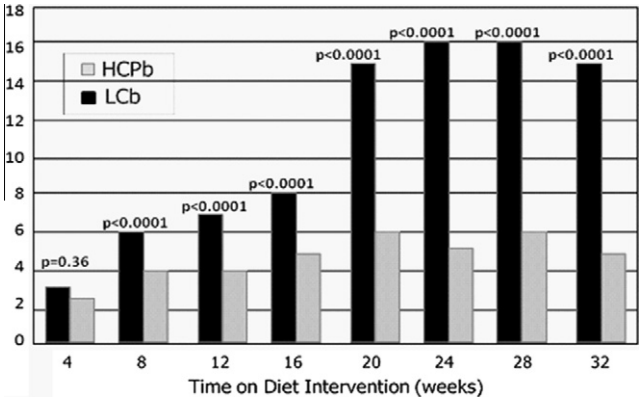


**Fig. 4.** Events of non-compliance by group and week.

study, starting at Week 8; despite this, weight loss was similar until Week 16. It is possible that despite the fact that there were more non-compliance events in the LCb group, the total number of such events remained relatively low, and did not exceed 8 such events through Week 16. After this point, however, the non-compliance event rate doubled, and remained high through the remainder or the study. It is conceivable that dietary non-compliance has a threshold effect, below which weight reduction is not inhibited, and above which weight regain occurs.

## 2. The paragraph referring to Table 3

The corrected version should read as follows:

At baseline, lipid profile (total cholesterol, triglycerides (TG), HDL, LDL) was similar between groups. By Week 16, TG values were significantly lower in the LCb group; however, by Week 32, TG and total cholesterol were significantly higher in the LCb group (Table 3).

## 3. Conflict of interest

Professor Jakubowicz is the author of "The Big Breakfast Diet". This book, based on clinical experience as an endocrinologist working with overweight patients, is directed at a general readership and certainly not at the scientific community. The book contains ideas based on years of practice, but is not evidence-based. The present study is the result of the research group's desire to test her ideas in a systematic fashion using the scientific method. To this end, we employed a randomized, treatment-controlled study design, which is the pinnacle design for establishing an evidence

base. Neither Prof. Jakubowicz nor any other member of the research team viewed using rigorous scientific methods to test a clinical observation as a potential conflict of interest. The study was not funded by the book's publisher and there is not and never was any financial association between the study and the book.

## 4. Corrected name of the affiliation of one of the authors

The word "Center" was omitted in typing.

The affiliation should be listed as: Ariel University Center of Samaria, Israel.