**Original Article**
CLINICAL TRIALS AND INVESTIGATIONS

Obesity

# Appetite-Related Responses to Overfeeding and Longitudinal Weight Change in Obesity-Prone and Obesity-Resistant Adults

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**Objective:** Appetite responses to 3 days of overfeeding (OF) were examined as correlates of longitudinal weight change in adults classified as obesity prone (OP) or obesity resistant (OR).

**Methods:** OP ($n=22$) and OR ($n=30$) adults consumed a controlled eucaloric and OF diet (140% of energy needs) for 3 days, followed by 3 days of ad libitum feeding. Hunger and satiety were evaluated by visual analog scales. Ghrelin and peptide YY (PYY) levels were measured during a 24-hour inpatient visit on day 3. Body weight and composition were measured annually for $4.0 \pm 1.3$ years.

**Results:** Dietary restraint and disinhibition were greater in OP than OR (mean difference: $3.5 \pm 1.2$ and $3.3 \pm 0.9$, respectively; $P<0.01$) participants, and disinhibition was associated with longitudinal weight change ($n=48$; $r=0.35$; $P=0.02$). Compared with the eucaloric diet, energy intake fell significantly in OR participants following OF ($P=0.03$) but not in OP ($P=0.33$) participants. Twenty-four-hour PYY area under the curve values increased with OF in OR ($P=0.02$) but not in OP ($P=0.17$) participants. Furthermore, changes in PYY levels with OF correlated with measured energy intake ($r=-0.36$; $P=0.01$).

**Conclusions:** Baseline disinhibition and PYY responses to OF differed between OP and OR adults. Dietary disinhibition was associated with 5-year longitudinal weight gain. Differences in appetite regulation may underlie differences in propensity for weight gain.

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## Study Importance

### What is already known?

- Approximately one-third of US adults maintain a normal body mass, despite living in an environment that promotes positive energy balance. Differences in the responses of appetite and energy intake following short periods of overfeeding (e.g., 3 days) may explain why some individuals are prone to obesity while others seem to be resistant.

### What does this study add?

- We measured indices of appetite regulation in response to 3 days of overfeeding (40% above usual intake) as compared with a eucaloric control condition in obesity-prone (OP) and obesity-resistant (OR) adult men and women.
- Our goal was to determine whether appetite responses to overfeeding correlate with longitudinal weight gain.
- Appetite ratings and appetite-related hormonal responses to overfeeding were not different between OP and OR adults, nor were these outcomes associated with weight gain. However, dietary restraint and disinhibition were greater in OP compared with OR, and dietary disinhibition was associated with weight gain in the combined group.

## Introduction

The prevalence of obesity has increased over a relatively short period (1). The modern obesogenic environment is likely a contributing factor (2-4). Periods of excessive energy intake (EI) with or without reductions in physical activity, leading to repeated periods of positive energy balance and weight gain, have become normal.

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Interestingly, despite living in the same environment, approximately one-third of Americans maintain a normal body weight. Understanding physiological and behavioral mechanisms that differ between obesity-prone (OP) and obesity-resistant (OR) individuals could provide valuable information on novel strategies to manage or prevent overweight/obesity. Our Energy Adaptations over Time Study (EATS) compared OP and OR adults classified by family and personal weight history and found that differences in substrate oxidation (5), neuronal responses to visual food cues (6,7), dietary restraint and disinhibition (8), and free-living physical activity following brief periods of overfeeding (OF) (9) may explain a propensity for weight gain.

The present analysis compares appetite-related hormones (ghrelin and peptide YY [PYY]), subjective appetite, and ad libitum EI following 3 days of OF versus 3 days of eucaloric (EU) intake in OP and OR adults. An additional aim was to determine whether acute responses to OF were associated with longitudinal weight change over 5 years of follow-up.

# Methods

## Participants

Characteristics of this cohort have been previously described (5,10). Nonobese (BMI < 30 kg/m$^2$), weight-stable (±5% for 3 months), young (25-35 years old) men and women classified as OR or OP were eligible for enrollment. OR participants defined themselves as "constitutionally thin" (not needing to exert effort to maintain their weight) and having no first-degree relatives with BMI > 30. OP participants identified themselves as having to exert a conscious effort to maintain their weight and reported at least one first-degree relative with BMI > 30. The Colorado Multiple Institutional Review Board approved the study protocol. Participants provided written informed consent prior to participation.

## Study design

Detailed information on the design has previously been published (5,9,11). Briefly, each participant completed baseline evaluations, including measurements of height, weight, and body composition via dual-energy x-ray absorptiometry (Hologic Discovery W; Hologic, Inc., Bedford, Massachusetts); the Three-Factor Eating Inventory Questionnaire (TFEQ) (12); and the Power of Food Scale (PFS) (13). Each participant then completed two 10-day controlled-feeding study phases in a randomized, crossover, counterbalanced design that were separated by at least 1 month. Each 10-day study phase consisted of (1) an outpatient 4-day EU run-in diet (study days 1-4), (2) a 3-day EU or OF (140% of estimated energy needs) diet period (study days 5-7, of which days 5 and 6 were outpatient stays and day 7 was an inpatient stay), and (3) an outpatient 3-day ad libitum feeding period, during which food intake was directly measured (Supporting Information Figure S1). Participants were invited to return for repeat dual-energy x-ray absorptiometry scans annually for 5 years. Participants who completed ≥ 1 year of follow-up are included in the present analysis.

*Run-in diet.* To ensure energy and macronutrient balance, participants consumed a controlled EU diet (20% protein, 34% fat, and 46% carbohydrates) for 4 days at the start of each study phase. The caloric value of the diet was individualized for each participant and determined by using measured resting metabolic rate and fat-free mass. Basal energy needs were multiplied by an activity factor of 1.4 to 1.65 based on 7 days of activity monitoring (pedometer). All food was prepared by the Colorado Clinical and Translational Research Center's (CTRC's) metabolic kitchen. Participants consumed breakfast on the unit each day, and the other meals were packaged for participants to take with them. Participants were instructed to consume only the food provided and were queried on adherence the following day.

*Study diets and inpatient stay.* After the 4-day EU run-in, participants consumed a controlled EU or OF diet (140% of baseline energy needs) in a randomized order for three subsequent days (study days 5-7). The macronutrient content was the same as that in the run-in diet. All food was prepared by the CTRC metabolic kitchen. Participants consumed breakfast on the unit each day, and the other meals were packaged for participants to take with them. Participants were instructed to consume only the food provided and were queried on adherence the following day. On the third day of the study diet (study day 7), participants were admitted to the inpatient CTRC at 7:00 AM in the fasting state for a 24-hour stay in a metabolic chamber. Breakfast, lunch, dinner, and a snack were provided at 7:30 AM (25% of daily EI based on the EU or OF diet), 12:00 PM (30% of daily EI), 5:00 PM (30% of daily EI), and 8:00 PM (15% of daily EI), respectively.

*Ad libitum diet.* After the 24-hour metabolic chamber stay, participants completed a 3-day, free-living, ad libitum feeding period (study days 8-10) using weigh and measure techniques to monitor EI. The amount of food offered to participants during the ad libitum portion was 125% of baseline energy needs. Participants were instructed to eat as much food as desired and to return unconsumed food. Breakfast was consumed on the unit each day, and the other meals were packaged for participants to take with them. All food for the 10-day study period was provided by the CTRC metabolic kitchen.

## Measurements

*Appetite ratings.* Immediately before and after each meal during the 3-day study diet phase (study days 5-7) and the subsequent 3-day ad libitum EI phase (study days 8-10), participants rated hunger, satiety, and prospective food consumption (PFC) using 100-mm visual analog scales (VAS) on a personal digital assistant (14).

*Appetite-related hormonal analysis.* At the start of the inpatient metabolic chamber stay (day 7), an intravenous catheter was inserted in the antecubital vein for blood sampling. Blood was drawn in the fasted state for ghrelin and PYY. After breakfast, blood was sampled at 8:30 AM and again every 30 minutes for 210 minutes. Then blood was sampled at clock times 1:00, 3:00, 5:00, 6:00, and 8:00 PM and then again at 2:00 and 7:00 AM the following day (day 8). The day 8 7:00 AM blood sample was also analyzed for leptin. Radioimmunoassay (RIA) was used to measure leptin (Millipore, Burlington, Massachusetts), PYY (Millipore), and total ghrelin (Millipore) levels by the CTRC Core Laboratory.

## Statistical analyses

Data were analyzed by using SAS version 9.4 (SAS Institute, Inc., Cary, North Carolina). $P < 0.05$ was considered statistically

significant. All data are presented as mean ± SE. VAS data were summarized as the daily average over the 3-day study diet phase (days 5-7) and over the 3-day ad libitum intake phase (days 8-10). Hormone data obtained on day 7 were summarized as the incremental area under the curve (iAUC) above or below baseline. iAUC was calculated by using trapezoidal reconstruction for the entire 24-hour chamber stay as well as for the daytime (8:00 AM-10:00 PM), nighttime (10:00 PM-6:00 AM), breakfast, lunch, and dinner (plus snack) periods separately. All outcomes were analyzed by using separate linear mixed models (SAS PROC MIXED procedure). Each linear mixed model consisted of group (OP or OR), diet (EU or OF), sequence (order of diet consumption), period (visit in which the diets were consumed), and interaction of group and diet as fixed effects and participants as random effects. Pearson correlation analysis was conducted among mean changes (Δ response = OF-EU condition) in ad libitum intake (days 8-10), mean changes in VAS scores (hunger, PFC, and satiety), mean changes in hormone levels (ghrelin, PYY, and leptin), and mean changes in baseline self-reported eating behavior scores (TFEQ and PFS). Mean changes in VAS scores, self-reported eating behavior scores, and hormone levels were also explored as correlates of longitudinal body weight and composition changes. The longitudinal data were expressed as rate of body weight change (RoWC) and rate of fat-mass change, calculated as the difference between the last follow-up time point minus baseline divided by the number of follow-up years.

**TABLE 1** Baseline participant characteristics and assessments of dietary restraint, disinhibition, and hunger

| | OP | OR | *P* value |
|---|---|---|---|
| ***n*, (% female)** | 22 (64) | 30 (47) | 0.23 |
| **Age, y** | 28.5 ± 0.6 | 28.0 ± 0.5 | 0.53 |
| **BMI** | 23.9 ± 0.5 | 20.5 ± 0.4 | < 0.001 |
| **Weight, kg** | 70.0 ± 2.0 | 63.5 ± 2.1 | 0.03 |
| **Fat mass, kg** | 18.4 ± 1.3 | 11.9 ± 0.5 | < 0.001 |
| **Fat-free mass, kg** | 51.2 ± 2.1 | 51.3 ± 2.0 | 0.98 |
| ***Baseline TFEQ and PFS*** | | | |
| **TFEQ, hunger** | 5.7 ± 0.7 | 5.1 ± 0.6 | 0.51 |
| **TFEQ, restraint** | 7.7 ± 1.1 | 4.2 ± 0.6 | 0.005 |
| **TFEQ, disinhibition** | 6.5 ± 0.8 | 3.2 ± 0.5 | < 0.001 |
| **PFS** | 46.3 ± 4.1 | 35.3 ± 1.5 | 0.007 |

Data presented as mean ± SE.
OP, obesity prone; OR, obesity resistant; PFS, Power of Food Scale; TFEQ, Three-Factor Eating Questionnaire.

# Results

## Study participants

Fifty-two participants (22 OP and 30 OR) completed both the EU and OF study periods. Four participants (2 OP and 2 OR) were omitted from the hormone analyses because of invalid plasma data. Correlational analyses were performed on individuals with valid data for both feeding conditions and ≥ 1 year of follow-up data ($n = 48$; 22 OP and 26 OR). Participant characteristics are presented in Table 1. OP participants had higher weight, BMI, and fat mass than OR participants. At baseline, OP participants reported greater levels of dietary restraint and disinhibition on the TFEQ and higher PFS scores than the OR participants (Table 1). Group differences in dietary restraint and disinhibition remained after statistical adjustment for BMI.

## Ad libitum EI

Figure 1 displays changes in EI and macronutrient intake (OF-EU) during the study diet phase (days 5-7) and each day of the ad libitum diet phase (days 8-10). Average EI over the 3-day ad libitum period

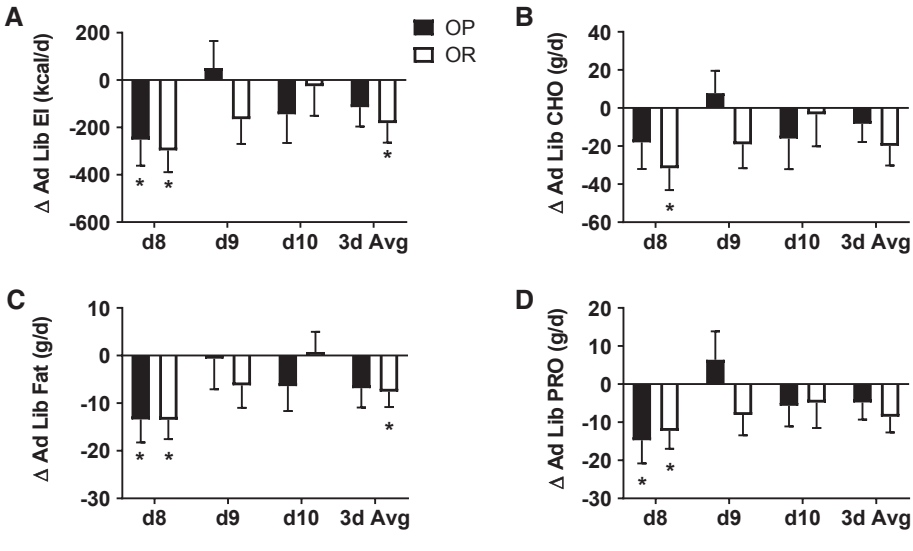


**Figure 1** Change in EI and macronutrient intake (OF-EU condition) for the study diet and ad libitum diet phases. (**A**) Total EI (kilocalories). (**B**) Carbohydrates (CHO) (grams). (**C**) Fat (grams). (**D**) Protein (PRO) (grams). *Significant within-group change in ad libitum intake following OF versus EU feeding phases. Δ, OF-EU condition; avg, average.

was not different between groups (diet×group interaction, $P=0.50$). However, when OP and OR participants were examined independently, the within-group decrease in EI following OF relative to the EU diet was significant in OR participants (−181.8±82.8 kcal, $P=0.03$), whereas the within-group decrease in EI was not significant in OP participants (−114.3±81.9 kcal, $P=0.33$). The largest decrease in EI for both groups occurred on day 8, immediately after the period of imposed OF.

## Hunger, PFC, and satiety

VAS measures summarized by day and as averages over each study phase are shown in Figure 2. OF decreased premeal hunger and PFC and increased postmeal satiety similarly in both groups during days 5 to 7 ($P<0.004$). After OF (ad libitum phase), VAS ratings of hunger, PFC, and satiety were similar to ratings given during the EU condition ($P>0.05$). Meal-specific (i.e., breakfast, lunch, and dinner+snack) appetite ratings during the first day of controlled feeding (EU or OF) and on the first day of the ad libitum period did not differ between groups (Supporting Information Figure S2).

## Twenty-four-hour ghrelin, PYY, and fasting leptin

Figure 3 depicts ghrelin and PYY concentrations over 24 hours during the inpatient visits. iAUC values (24 hours, during the day, at night, and per meal) are shown in Figure 4. The mean ghrelin iAUC over 24 hours was −11.3%±1.2% lower in OP participants and −6.2%±1.6% lower in OR participants following OF, but the group×diet interaction was not significant ($P=0.91$). Postmeal suppression of ghrelin during the daytime was also not different between groups (diet×group interaction, $P=0.84$).

Over 24 hours, the PYY iAUC was 75%±9.4% higher during OF in OR participants ($P=0.02$) and 41.1%±7.6% higher in OP participants, with no difference between groups (diet×group interaction, $P=0.39$). However, when OP and OR participants were examined independently, the within-group increase in PYY levels during OF was significant in the OR group ($P=0.02$) but not in the OP group ($P=0.17$). There were also trends for significant between-group differences for the PYY responses to breakfast (diet×group interaction, $P=0.05$) and dinner (diet×group interaction, $P=0.09$). During OF, the OP group had an increase in PYY levels relative to the EU condition, whereas PYY levels were not different between conditions in the OR group at breakfast. In contrast, the PYY

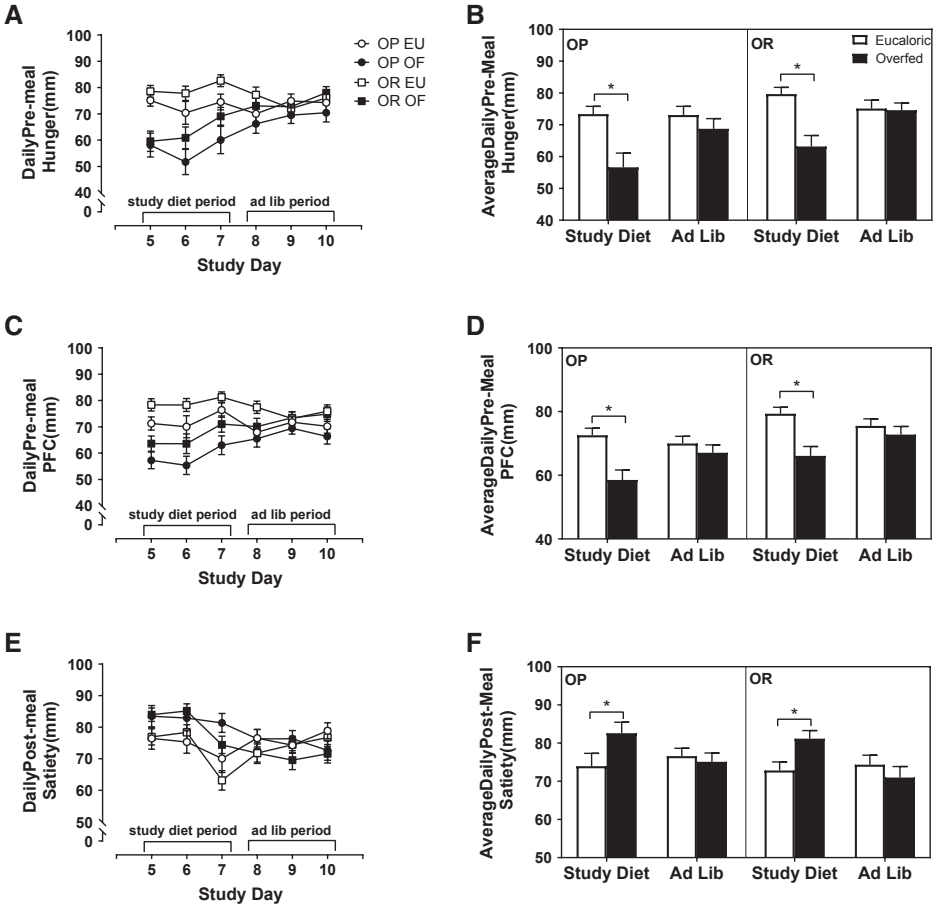


**Figure 2** Time course of visual analog scale measures of (**A**) premeal hunger, (**C**) premeal prospective food consumption (PFC), and (**E**) postmeal satiety in OP and OR adults studied for 3 days under controlled EU and OF conditions followed by a 3-day ad libitum diet. Participants consumed the study diet (EU or OF) on days 5 to 7 and the ad libitum diet on days 8 to 10. Average values for (**B**) hunger, (**D**) PFC, and (**F**) satiety during the study diet and the ad libitum diet period. *Significant diet effect, $P<0.05$.

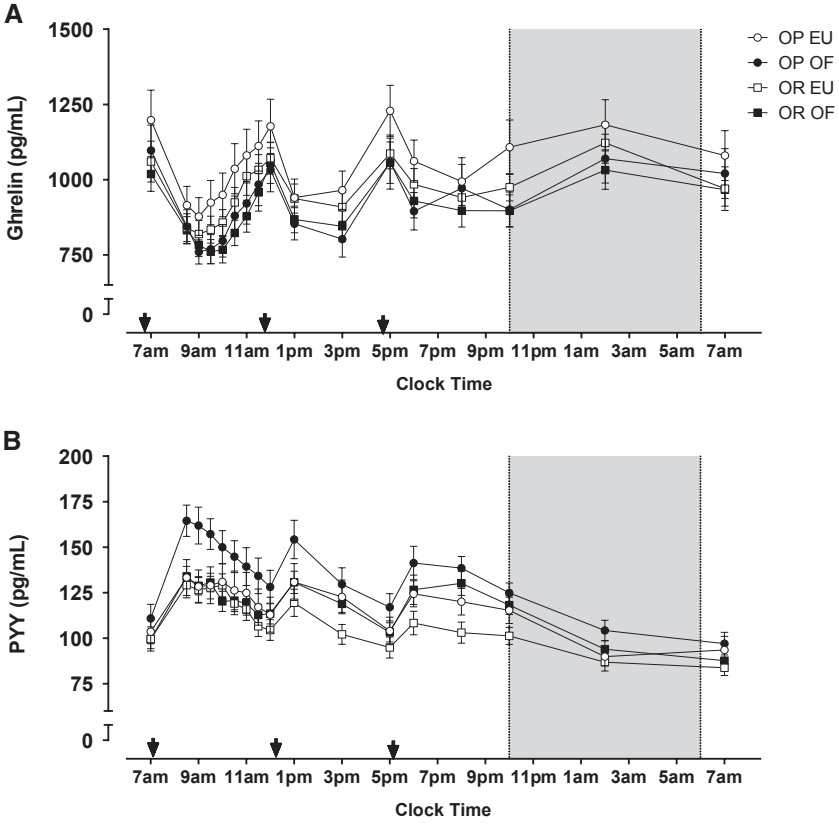


**Figure 3** Twenty-four-hour (**A**) ghrelin and (**B**) PYY responses to OF (compared with EU control diet) in OP and OR adults. Arrows indicate breakfast, lunch, and dinner. Shaded area indicates sleep opportunity in the room calorimeter. PYY, peptide YY.

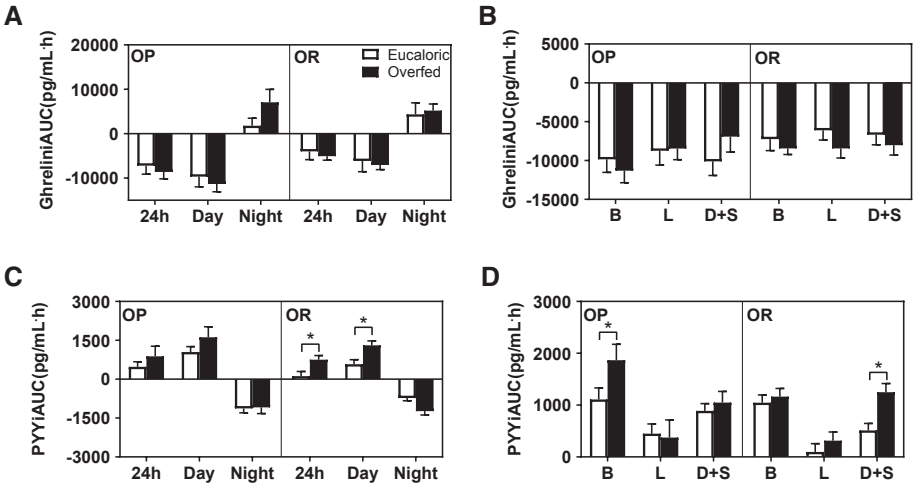


**Figure 4** Summary measures (incremental area under the curve [iAUC] above baseline) for ghrelin and PYY (**A,C**) over 24 hours, during the day, and at night and (**B,D**) during feeding periods in OP and OR adults studied for 3 days under controlled EU and OF conditions. *Significant diet effect, $P<0.05$. B, breakfast; D+S, dinner plus snack; L, lunch.

response to dinner was increased relative to EU feeding in OR participants but not in OP participants. The trends for between-group differences in PYY at breakfast and dinner remained after statistical adjustment for BMI.

Fasting leptin concentrations were higher in OP compared with OR participants under both experimental conditions ($P$ for group < 0.001), but these differences disappeared after adjustment for fat mass ($P$ for group = 0.61; Supporting Information Figure S3). Fasting leptin concentrations during OF were not significantly different from those under EU conditions in either group ($P$ for diet effect = 0.13).

## Correlates of ad libitum EI and prospective weight change

Bivariate correlations among baseline TFEQ, hormone, and VAS responses to OF and ad libitum EI are shown in Table 2. Scatter plots showing

**TABLE 2** Bivariate correlations among measures of baseline dietary restraint, subjective and hormonal responses to OF, and ad libitum EI over a period of 3 days following OF

| | Δ Ad libitum EI (day 8) | Δ Ad libitum EI (day 9) | Δ Ad libitum EI (day 10) | Δ Ad libitum EI (average days 8-10) |
|---|---|---|---|---|
| TFEQ, hunger | **0.38 (*P* = 0.009)** | 0.24 (*P* = 0.11) | 0.22 (*P* = 0.14) | **0.39 (*P* = 0.007)** |
| TFEQ, restraint | 0.11 (*P* = 0.45) | 0.03 (*P* = 0.87) | 0.07 (*P* = 0.63) | 0.11 (*P* = 0.46) |
| TFEQ, disinhibition | 0.15 (*P* = 0.32) | 0.20 (*P* = 0.17) | 0.06 (*P* = 0.70) | 0.20 (*P* = 0.18) |
| PFS | −0.06 (*P* = 0.69) | 0.10 (*P* = 0.49) | −0.11 (*P* = 0.45) | −0.02 (*P* = 0.87) |
| **Δ Premeal hunger rating** | **0.32 (*P* = 0.03)** | 0.11 (*P* = 0.44) | −0.05 (*P* = 0.75) | 0.16 (*P* = 0.26) |
| **Δ Premeal PFC rating** | **0.40 (*P* = 0.005)** | 0.17 (*P* = 0.24) | 0.09 (*P* = 0.56) | **0.28 (*P* = 0.05)** |
| **Δ Postmeal satiety rating** | 0.05 (*P* = 0.72) | −0.17 (*P* = 0.25) | −0.19 (*P* = 0.19) | −0.14 (*P* = 0.35) |
| **Δ 24-h Ghrelin iAUC** | −0.12 (*P* = 0.39) | 0.05 (*P* = 0.75) | −0.10 (*P* = 0.48) | −0.08 (*P* = 0.60) |
| **Δ 24-h PYY iAUC** | −0.26 (*P* = 0.07) | −0.20 (*P* = 0.17) | **−0.29 (*P* = 0.05)** | **−0.36 (*P* = 0.01)** |
| **Δ Fasting leptin** | −0.13 (*P* = 0.36) | 0.04 (*P* = 0.77) | 0.13 (*P* = 0.39) | 0.04 (*P* = 0.79) |

Significant differences are indicated in bold.
Δ, overfed-eucaloric condition; EI, energy intake; iAUC, incremental area under the curve; OF, overfeeding; PFC, prospective food consumption; PFS, Power of Food Scale; PYY, peptide YY; TFEQ, Three-Factor Eating Questionnaire.

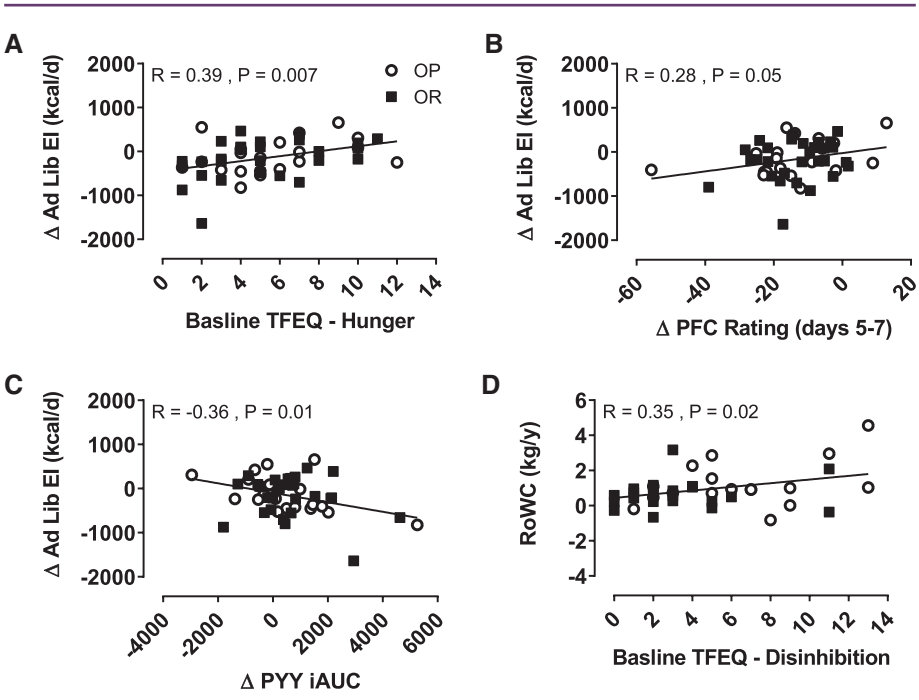


**Figure 5** Correlates of ad libitum energy intake (EI) and **(A)** baseline hunger, **(B)** change in prospective food consumption (PFC), and **(C)** change in PYY iAUC and of **(D)** prospective weight gain and baseline disinhibition. Δ Ad libitum intake is expressed as the average intake during study days 8 to 10. Δ, OF-EU condition; RoWC, rate of weight change; PYY, peptide YY.

**TABLE 3** Bivariate correlations among measures of baseline dietary restraint, subjective and hormonal responses to short-term OF, and prospective weight change

| | RoWC (kg/y) | RoFMC (kg/y) |
|---|---|---|
| Baseline TFEQ, hunger | 0.19 ($P=0.24$) | −0.06 ($P=0.69$) |
| Baseline TFEQ, restraint | −0.11 ($P=049$) | −0.29 ($P=0.07$) |
| Baseline TFEQ, disinhibition | **0.35 ($P=0.02$)** | 0.05 ($P=0.75$) |
| Baseline PFS | 0.17 ($P=0.30$) | −0.03 ($P=0.88$) |
| **Δ Premeal hunger rating** | 0.08 ($P=0.63$) | −0.01 ($P=0.93$) |
| **Δ Premeal PFC rating** | 0.05 ($P=0.77$) | −0.11 ($P=0.47$) |
| **Δ Postmeal satiety rating** | 0.21 ($P=0.18$) | 0.20 ($P=0.20$) |
| **Δ Ad libitum EI (days 8-10)** | 0.21 ($P=0.16$) | 0.17 ($P=0.26$) |
| **Δ 24-h Ghrelin iAUC** | −0.14 ($P=0.36$) | −0.07 ($P=0.66$) |
| **Δ 24-h PYY iAUC** | −0.20 ($P=0.19$) | −0.02 ($P=0.88$) |
| **Δ Fasting leptin** | 0.21 ($P=0.20$) | −0.12 ($P=0.45$) |

Significant differences are indicated in bold.
Δ, overfed (OF)-eucaloric condition; EI, energy intake; iAUC, incremental area under the curve; PFC, prospective food consumption; PFS, Power of Food Scale; PYY, peptide YY; RoFMC, rate of fat-mass change; RoWC, rate of weight change; TFEQ, Three-Factor Eating Questionnaire.

significant associations are presented in Figure 5. Individuals with higher TFEQ hunger ratings at baseline consumed more energy during the ad libitum period following the OF condition (Table 2, Figure 5). Similarly, individuals reporting increased hunger and PFC during OF consumed the most energy during the ad libitum diet. A greater increase in the PYY response to OF was associated with lower ad libitum intake during the subsequent 3 days ($r=-0.36$, $P=0.01$; Table 2, Figure 5).

The OP and OR groups gained 3.5±0.8 kg and 2.6±0.6 kg of body weight, respectively, with no differences between groups over 4.0±1.4 years of follow-up ($P=0.70$). These changes in body weight corresponded to a 2.4±0.7-kg increase in fat mass in OP participants and a 1.9±0.4-kg increase in fat mass in OR participants during the follow-up period. Bivariate correlations between the hormone responses to OF and prospective weight changes are shown in Table 3. None of the responses to OF that were explored were significant predictors of body weight or composition changes over the follow-up period. Higher TFEQ disinhibition ratings at baseline were associated with a greater RoWC, which tended to be driven by the OP group (correlation coefficient for the combined group=0.35, $P=0.02$; correlation coefficient for the OP group=0.39, $P=0.12$; correlation coefficient for the OR group=0.11, $P=0.61$; Table 3, Figure 5).

## Discussion

This study examined appetite-related hormonal and behavioral responses to short-term OF in adults classified as OP and OR and tested whether acute responses were associated with long-term weight gain. Findings indicate differences in some of these variables between OP and OR individuals that may be involved in the propensity for weight gain. Specifically, in response to OF, within-group 24-hour PYY values were significantly increased in the OR but not in the OP group, and average EI for the 3-day ad libitum feeding period was significantly reduced in OR but not in OP participants. In addition, although OP and OR adults gained similar amounts of body mass over 5 years of follow-up, higher baseline dietary disinhibition was associated with greater RoWC, an effect that was driven primarily by the OP group.

Consistent with our previous study (8), we found that OP adults demonstrated greater dietary restraint and disinhibition (12) as well as a greater drive to consume palatable foods in the absence of physiological hunger (13). Disinhibition and hedonic hunger have been positively correlated with obesity and weight gain (15-19) in other studies. The relationship between dietary restraint and both current body weight and longitudinal weight change is less clear. Some studies have shown a positive relationship between dietary restraint and body mass (20,21); others have reported an inverse association or no relationship (17,22-26). Discrepancy between our findings and other investigations is likely due to specific inclusion criteria. Our group of OP individuals were normal weight adults who reported exerting conscious effort to maintain their weight. The combination of greater dietary restraint with greater disinhibition is unique and suggests that despite making efforts to limit caloric intake, OP adults might be more likely to overeat when confronted with certain foods, situations, or emotional states.

Ad libitum EI decreased in response to the OF versus EU diet in both groups on day 8. This indicates that caloric compensation occurs in most individuals to restore energy balance following short-term OF. However, the 3-day average ad libitum EI was significantly reduced following OF only in the OR group, not the OP group, suggesting that OR individuals may be better able to compensate for the energy surplus and thus better able to maintain their weight over time. However, this hypothesis was not supported by the data because OP and OR participants had similar RoWC. EI during the ad libitum feeding period was also not related to RoWC during follow-up. The lack of a relationship could be due to the ad libitum feeding protocol employed. OF by 40% above baseline energy needs may be an insufficient stimulus to reveal adaptive responses that predict future weight change. However, our finding is in agreement with two prior investigations that, despite providing unlimited access to 40 participant-selected foods via vending machine technology (27,28), also found that total ad libitum EI was not associated with weight gain over 6 months to 11 years of follow-up (27,28). Interestingly, consumption of specific foods (i.e., those high in fat and simple sugars) was correlated with weight gain over time (27,28). In these trials, using a vending machine feeding paradigm, participants consumed approximately 150% of their basal energy needs, with an SD of approximately 46%. Thus, future trials evaluating how ad libitum EI and food choice influence weight gain may need to provide opportunity for consumption of very high EI.

We also evaluated appetite before and after each meal during the OF and EU phases as well as the ad libitum period. We found no differences between OP and OR groups. For the entire sample, OF led to a significant decrease in premeal hunger and PFC and increased postmeal satiety ratings. These results are also in line with our prior investigation of 1 day of an OF or EU diet in OP and OR adults (8), providing further evidence that self-reported appetite is not predictive of predisposition to obesity. We hypothesized that OR individuals would sense OF more rapidly than OP individuals. However, no differences between groups were noted on day 5 or day 8 (see Supporting Information Figure S2) for appetite ratings. Despite no between-group differences in measures of appetite, individuals reporting greater hunger and PFC in response to OF consumed a greater number of calories during the ad libitum phase following OF versus the EU condition.

We were also interested in determining whether PYY and ghrelin levels would be altered in response to OF between OP and OR groups. Overall,

OF did not result in changes to PYY or ghrelin levels compared with the EU condition, nor were group differences in the PYY response to OF detected. However, there was a statistically significant within-group increase in 24-hour PYY iAUC in the OR group. This increase was driven by the increased PYY response following the dinner meal and nighttime snack. Furthermore, a greater PYY response following OF was associated with lower ad libitum EI, as previously demonstrated (29). These data are consistent with the idea that OP individuals may be more susceptible to reduced satiety and greater EI during the evening. Evidence has consistently shown an association between EI later in the day and increased BMI (30).

Our group previously reported no differences in fasting or postprandial PYY responses to 1 day of OF between OP and OR adults as well as no overall diet effect of OF on PYY levels (14). Although, interestingly, in that prior study, underfeeding did result in a significant reduction in PYY levels (14). It is possible that the degree of OF in our trials was not great enough to elicit significant changes in PYY levels. Other investigators reported an increase in fasting PYY levels following 7 days of OF at 170% of energy needs in men (31). Furthermore, the increase in fasting PYY levels in that trial was not related to adiposity status (31), which is in agreement with our finding that PYY levels do not differ between OP and OR phenotypes. Of note, a small, free-living, 4-week OF intervention comparing women with constitutional thinness (women with BMI similar to that in patients with anorexia nervosa but without an eating disorder) and normal weight controls found that constitutionally thin participants demonstrated an increase in postprandial PYY levels following OF, whereas the normal weight controls experienced a decrease in postprandial PYY levels (32). Thus, in rare cases of extreme obesity resistance, gut peptides may play a role in the preservation of a thin phenotype. In our OR participants who reported not having to exert effort to maintain a normal BMI, gut peptides appear to be of less importance. Furthermore, the PYY response to short-term OF was not associated with longitudinal weight change, further suggesting that this gut hormone may not be predictive of long-term weight gain.

Contrary to our hypothesis, ghrelin was not influenced by OF in either group. This is surprising because we expected OF to decrease ghrelin concentrations and thus promote a reduction in subsequent EI. Inconsistent findings in ghrelin responses to OF exist in the literature. We previously found a reduction of the postprandial ghrelin iAUC in response to 1 day of OF in OP and OR adults (14), and Robertson et al.(33) demonstrated greater postprandial ghrelin suppression in response to an oral fat tolerance test after 3 weeks of high-fat feeding in a small ($N=6$) study of healthy men (34). Other investigations have also demonstrated no change in fasting or post-Oral Glucose Tolerance Test ghrelin following short-term (3 days) OF (35,36). However, fasting acylated ghrelin levels have been shown to increase following short-term OF (34), further complicating our understanding of ghrelin's role in maintaining energy balance. Discrepant outcomes are likely related to different OF protocols, the form of ghrelin (acylated vs. deacylated vs. total) measured, and the health status of participants. In addition to ghrelin's appetite-specific roles, it is also implicated in glucose regulation, and thus responses to short-term OF could be more related to glycemic control than appetite regulation (37,38).

Based on prior findings from our group and others, we hypothesized that RoWC would be greater in OP versus OR adults. With 5 years of follow-up, no statistically significant difference in RoWC or body composition was detected between these groups. However, it is important to note that the OR group entered the study with lower BMI and therefore maintained a lower BMI through follow-up. This could have important implications for cardiometabolic health outcomes and weight gain trajectories beyond our follow-up period. Although no differences in RoWC between the groups were apparent, baseline disinhibition was positively associated with RoWC, an effect driven by the OP group. Our findings add information on how dietary constructs prospectively influence weight gain and extend prior retrospective analyses. Specifically, Hays and Roberts (15) previously reported that higher levels of disinhibition were associated with approximately 22 kg of weight gain over a 20-year period. This trial enrolled older women and asked them to self-report their body mass at six prior age ranges. Interestingly, they evaluated subscales of the TFEQ and found that "habitual disinhibition," which is the susceptibility to overeat in response to daily life circumstances (e.g., nearly constant access to energy-dense, palatable foods) was the greatest predictor of weight gain. Collectively, these data indicate that easy-to-administer questionnaires may provide valuable insight on risk of future weight gain. Furthermore, targeting disinhibition could be an effective intervention approach.

We acknowledge a number of limitations to this study. First, OP and OR phenotypes are based on self-report, and these groups differed in body mass and composition at study onset. Second, measurements of appetite ratings occurred only before and after meals and did not overlay the blood sampling time points during the inpatient stay. Third, blood sampling occurred most frequently after the breakfast meal on day 8 and was not conducted at uniform times after each meal. Similarly, the nighttime blood sampling period had only two collections to avoid disrupting participants' sleep. Fourth, we measured total forms of ghrelin and PYY and therefore are unable to comment on how the active forms of these hormones may be altered. Fifth, we acknowledge that the significant increase in 24-hour PYY levels and decrease in 3-day ad libitum EI following OF in the OR group versus no significant changes in the OP group is not synonymous with between-group differences and may represent a difference in nominal significance (39). Thus, we cautiously present these findings. However, it is important to note that the trial was not powered to detect significant group by time interactions in this secondary analysis. Finally, we had a reduced sample size for the longitudinal weight change analysis, which could have reduced our ability to detect significant relationships.

## Conclusion

Our data indicate that OP adults exhibited greater dietary disinhibition than OR adults and that greater baseline disinhibition was associated with greater weight gain over 5 years of follow-up. Furthermore, OF resulted in significant increases in PYY iAUC and reductions in 3-day ad libitum EI in the OR group but not in the OP group, providing information on potential physiological differences in these phenotypes. However, no difference in longitudinal weight gain occurred between these groups. Trials with larger samples for longitudinal follow-up and examination of other behavioral and physiological predictors of weight change will be required to confirm these relationships and determine whether OP and OR classifications are predictive of weight gain. O

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**Author contributions:** TH, CAR, and ET analyzed and interpreted the data and wrote the first draft of the manuscript. ZP assisted with data analysis and interpretation. AB assisted with interpretation of the data and writing. EHK conducted the experiments. MAC and DHB conceived and conducted the experiments, interpreted the data, and edited the final draft of the manuscript. All authors were involved in writing the article and had final approval of the submitted and published versions.

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