A PILOT STUDY OF GUT-BRAIN SIGNALING AFTER OCTREOTIDE THERAPY FOR UNINTENTIONAL WEIGHT LOSS AFTER ESOPHAGECTOMY

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ABSTRACT

Background

Recurrence-free patients after esophageal cancer surgery face long-term nutritional consequences, occurring in the context of an exaggerated post-prandial gut hormone response. Acute gut hormone suppression influences brain reward signaling and eating behavior. This study aimed to suppress gut hormone secretion and characterize reward responses and eating behavior among post-esophagectomy patients with unintentional weight loss.

Methods

This pilot study prospectively studied post-operative patients with ≥10% body weight loss (BWL) beyond one year who were candidates for clinical treatment with long-acting Octreotide (LAR). Before and after four weeks of treatment, gut hormone secretion, food cue reactivity (functional MRI), eating motivation (progressive ratio task), *ad libitum* food intake, body composition, and symptom burden were assessed.

Results

8 patients (7 male, age: mean±SD 62.8±9.4 years, post-operative BWL: 15.5±5.8%) participated. Octreotide LAR did not significantly suppress total post-prandial plasma GLP-1 response at four weeks (P=0.08). Post-prandial symptom burden improved after treatment (Sigstad score median(range): 12(2-28) vs. 8(3-18), P=0.04), but weight remained stable (Pre:68.6±12.8kg vs. Post:69.2±13.4kg, P=0.13). There was no significant change in brain reward system responses, during evaluation of high-energy or low-energy food pictures, nor their appeal rating. Moreover, treatment did not alter motivation to eat (P=0.41) nor *ad libitum* food intake(P=0.46).

Conclusion

The protocol used made it feasible to characterize the gut-brain axis and eating behavior in this cohort. Inadequate suppression of gut hormone responses four weeks after Octreotide LAR administration may explain the lack of gut-brain pathway alterations. A higher dose or shorter inter-dose interval may be required to optimize the intervention.

Keywords: Esophagectomy; weight loss; gut-brain axis; enteroendocrine signaling; eating behavior; fMRI; food reward

INTRODUCTION

Unintentional weight loss and gastrointestinal symptoms associated with malnutrition and impaired functional recovery are serious and common concerns for patients after esophagectomy, even among those who remain free from recurrent disease in the long-term (1-4). In addition to weight loss, malnourished patients may have sarcopenia and osteopenia (5-7). These issues accentuate the psychosocial burden experienced by patients post esophagectomy, impairing physical function and long-term health-related quality of life (HRQL) (8-10). Though there are myriad drivers of weight loss following esophageal cancer surgery, the impact of exaggerated post-prandial gut hormone signaling on appetite and satiety has emerged as a novel modifiable factor (11). This has more thoroughly been researched in bariatric surgery contexts and is relatively understudied post-esophagectomy (12-14). However, targeted therapies, specifically modulating the gut-brain axis by suppressing gut hormone secretion, such as somatostatin analogues, have considerable theoretical rationale.

Scientific understanding of neural and hormonal pathways linking the gut to the brain is evolving. The dopaminergic mesolimbic pathway is the cornerstone of hedonic signaling, which together with hypothalamic homeostatic pathways control food intake and eating behavior, and this is influenced by feeding status, energy balance, perceived momentary value of food, and past learned experiences (15-19). Taking a reductionist approach, eating behavior can be viewed as the sum of appetitive and consummatory behaviors, with the former representing, in this case, a positive motivation to seek food ('wanting'), and the latter, the actual consumption of food, which is intimately linked with, and driven by, anticipatory and actual food 'pleasure' or reward ('liking') (20, 21). Though methodologically challenging, it may be possible to measure appetitive behavior through techniques validated in bariatric research. Functional MRI and progressive ratio tasks (12), circumvent the limitations associated with self-reported methods, which are hypothesis-generating but prone to biases (22-24). Direct measurements of food intake and preferences represent a more robust and accurate approach to

characterizing consummatory behavior (25, 26). Measuring both appetitive and consummatory components of eating behavior in parallel with gut hormone signaling may provide insight into changes in gut-brain signaling, and the impact of potential therapeutic strategies.

In the context of esophageal cancer surgery, the mechanisms by which gut hormones, such as glucagon-like peptide-1 (GLP-1) may influence eating behavior, symptomatology, and nutrition after surgery are becoming increasingly understood. Acute suppression of the exaggerated satiety gut hormone response using the somatostatin analogue Octreotide can increase *ad libitum* food intake by 50 per cent (27). Octreotide also increased motivation to eat in the acute setting, inducing a stronger desire to obtain a sweet-fat stimulus.(28) Several aspects of gut-brain signaling, however, remain unexplored in this operative context. First, the association between gut hormone suppression therapy post-esophagectomy and anticipatory food reward on fMRI is unknown. Second, how a short course of Octreotide therapy affects gut hormone signaling and eating behavior has not been reported. In various clinical contexts associated with weight loss and dumping syndrome, long-acting Octreotide (Octreotide LAR), which can reduce post-prandial symptoms and induce weight gain, has been used for decades (29, 30). However, there is a dearth of evidence regarding how best to employ this treatment paradigm to ameliorate excessive post-operative weight loss, currently limiting the viability of this targeted treatment approach. Parallel assessment of gut hormone signaling, food reward response, and eating behavior has not been performed previously but such a methodological approach may facilitate both a greater understanding of post-esophagectomy physiology and the therapeutic benefit of long-acting somatostatin analogue therapy.

It is within this context that this pilot study sought to: a) comprehensively characterize the gut-brain signaling pathway in a post-esophagectomy cohort, b) assess gut hormone and brain reward system food cue reactivity associated with a 4-week course of Octreotide LAR therapy, and c) determine the relationship between eating behavior and Octreotide LAR treatment.

MATERIALS AND METHODS

Participants

We recruited patients who were disease-free at least 1 year post curative intent esophagectomy with gastric conduit reconstruction for esophageal cancer, who had ≥10% unintentional weight loss and were scheduled to receive Octreotide LAR as a trial of therapy to improve weight. All patients had a significantly raised Sigstad score (>7) or persistent lack of appetite. First treatment dose was given in the outpatient clinic, with a shared decision made with each patient based on whether to continue based on treatment response after four weeks. Response was assessed using self-perceived improvement based on questionnaire scores as well as objective changes in appetitive behaviour and weight. Exclusion criteria included: nutritional compromise due to overt issues such as anastomotic stricture with significant dysphagia or significant malabsorption of any cause, the presence of neuropsychiatric illness, the presence of alcohol or other substance misuse disorders, contraindications to the use of Octreotide LAR.

Study Design and Protocol

This study was approved by the regional research ethics committee (2017-07 (6)) and it was undertaken in accordance with the principles of ICH Good Clinical Practice with written, informed consent obtained from all participants. The study was prospectively registered on ClinicalTrials.gov (NCT03377660). Participants were studied on two occasions, before and four weeks after an intramuscular dose of 10mg Octreotide LAR (Figure 1), as used previously to treat dumping syndrome in the post-gastrectomy context [Penning, 2005 #7], which was accompanied by a prophylactic course of pancreatic enzyme replacement therapy. At each visit, participants attended the Clinical Research Facility at 08:45 having completed a 12-hour fast, excluding water and regular medications. The protocol had four components, all of which were completed on the same day. These included (a) standardized mixed meal challenge, (b) functional MRI tasks, (c) progressive ratio task, and (d) *ad libitum* buffet meal.

Mixed meal challenge

Venous blood samples were drawn before, and at 30, 60, 90, 120, and 150 minutes after a 400 kcal standard semi-solid mixed meal (160 g (184 mL): 61.0% fat, 6.7% protein, 32.3% carbohydrate). Using a 10 cm visual analogue scale recorded at each blood sampling time point, temporal patterns of hunger, nausea, desire to eat, prospective food consumption, fullness, and sleepiness were measured. A composite appetite score was also calculated based on VAS scores, based on the following formula: [hunger + (100 – fullness) + desire to eat + prospective food consumption] / 4 (31).

Each participant completed European Organization for Research and Treatment of Cancer (EORTC) HRQL questionnaires (General HRQL: QLQ-C30, and cancer-specific sections: QLQ-OES18 and QLQ-OG25) (32, 33), as well as a Sigstad post-prandial dumping syndrome questionnaire and a modified gastrointestinal symptom rating scale (32). The Sigstad score is a questionnaire utilized in the diagnosis and assessment of post-operative dumping syndrome, with a score greater than seven indicative of clinically significant dumping syndrome. Body weight and body composition were assessed using bio-electrical impedance analysis (BIA, Seca 515, Seca GMBH & Co., Hamburg, Germany) with participants wearing light clothing, post voiding of urine, as per best practice (34, 35).

Functional MRI Tasks

Study participants then underwent both structural and functional magnetic resonance imaging (fMRI) scanning over a 60 minute period, from 180 – 240 minutes, following a previously described protocol (12, 36-38). During this period they performed a food picture evaluation fMRI task in which they rated the appeal of pictures of high-energy foods, low-energy foods and objects (12, 36-38), followed by a control auditory-motor-visual (AMV) task (38). For the food picture evaluation fMRI task, the effects of Octreotide LAR treatment on blood oxygen level dependent signal (BOLD) was examined by both *a priori* function region of interest (fROI) analysis (nucleus accumbens, caudate, putamen,

amygdala, anterior orbitofrontal cortex (OFC), anterior insula) and whole brain analysis for the following picture contrasts: high-energy > low-energy food, high-energy food > object, low-energy food > object, nor any food (high-energy or low-energy food) > object. A detailed description of the fMRI methodology is included in the Supplementary methods(39).

Progressive Ratio Task

Participants were asked at each visit to complete a computer-based progressive ratio task (PRT) following the fMRI, as per a previously described protocol [Goldstone, 2016 #2957;Miras, 2012 #3625]. This validated paradigm involves using a sweet-fat stimulus to gauge motivation or desire to eat. Each session lasted for up to 15 minutes (or upon cessation of the task by the participant themselves) and was performed once before and once after treatment with Octreotide LAR. Participants were asked to click the computer mouse in order obtain the candy reward reinforcer. The number of required clicks to receive the reward increased from 10, at the first stage, in a geometric increment of two (10, 20, 40, 80, 160, 320 clicks etc.), with the final completed increment referred to as the 'PRT breakpoint'. At each stage, the computer program instructed participants to ingest a single reward. It then urged participants to continue clicking the mouse, if they so desired, to obtain the next reward. They could continue clicking or stop at any point when the arduousness of the task outweighed the perceived value of the food reward. Visual analogue scales were completed, and blood samples taken, immediately before the PRT and after the subsequent buffet meal.

Ad libitum buffet meal

The final test was a buffet meal paradigm, conducted in a research kitchen, which used a direct measures approach to assessing macronutrient intake and preference based upon a method created and validated by Geiselman *et al* (40). When recruited to the study, participants completed a screening food preference questionnaire, which was used to inform the 18 food items that comprised the personalized participant buffet meal for each visit. The selected foods represented six macronutrient

groupings, of which three were high-fat and three were low-fat. Within each trio, the carbohydrate (complex and simple) and protein content varied. Quantities of each of the 18 foods were provided in excess to ensure there was no limit to *ad libitum* consumption, which could introduce bias. Participants were not aware that macronutrient type and quantity ingestion was being measured, with foods weighted prior to testing. Instead, participants were informed that in order to optimize the post-prandial stimulation of gut hormones, they should consume the foods they liked until they were comfortably satiated. This 45 minute study stage was followed by a final blood draw and VAS score completion. The foods remaining were then weighed covertly to ascertain the exact quantity consumed.

Plasma analysis

Once drawn, blood samples were placed on ice and carried to the laboratory in an adjacent room. They were immediately centrifuged at 2500 rpm at 4°C for 10 minutes, with plasma then stored at -80 °C to minimize sample degradation. Plasma total GLP-1 levels were measured by sandwich enzyme-linked immunosorbent assay (ELISA) (GLP-1 Total ELISA, Merck Millipore, Darmstadt, Germany). The assay used was sensitive, validated to detect above 1.5 picomolar (pM) with a 50µL sample, and specific, having no cross-reactivity with GLP-2, GIP, glicentin, or oxyntomodulin. The intra- and inter-assay co-efficient of variation were <2%, and <12%, respectively. Plasma glucose and insulin were measured using an automated analyzer (ALINITY, Abbott Laboratories, IL, USA).

Statistics

Power calculations

This was a pilot study with the principal aim to determine feasibility of the protocol and effect size of the outcomes. When planning the study we used the PRT breakpoint as primary outcome as our previous data demonstrated that the difference in the response of matched pairs to Octreotide is normally distributed and is 88 with standard deviation of 47 (28). Were these values used, with a

significance level of 0.05 and 0.9 power, then 5 participants would be needed to detect a difference with Octreotide LAR treatment.

Statistical analysis

GraphPad Prism (version 8.3) for macOS (GraphPad software (San Diego, CA, USA)), was used for data analysis. As per EORTC recommendations, a linear transformation of EORTC questionnaire responses into numeric values ranging from 0-100 was conducted, with rising scores indicating increased symptom severity or improved quality of life scores. Data were reported as mean ± standard deviation, or median (range), as appropriate, unless otherwise specified. Area under the curve (AUC) was calculated using the trapezoidal rule. Inferential testing for within-group univariate comparisons involved paired Student's *t* or Wilcoxon signed rank tests, as appropriate. To test for significant differences within individual variables over multiple timepoints, one-way repeated measures analysis of variance (ANOVA) with *post-hoc* Bonferroni's multiple comparisons test employed, unless otherwise stated. In the case of two or more variables followed over time, two-way repeated measures ANOVA with *post-hoc* Bonferroni multiple comparisons test used. Continuous variable inter-relationships were determined using simple linear regression. Effect sizes are described as mean difference [95% confidence intervals (CI)], and Cohen's d values. Statistical analyses were two-tailed, and *P*-values below an alpha level of 0.05 were deemed statistically significant.

RESULTS

Cohort characteristics

The study cohort was demographically representative of a typical post-operative Western esophageal cancer population, with a mean age of 63 years, 7 (87.5%) males, and 6 with adenocarcinoma (75%) (Table 1). While pre-operatively participants tended to be overweight with a mean BMI of 27.2 kg/m^2, this fell to 22.4 kg/m^2 by the first study visit. At a median of 23.6 months post-operatively, the average weight loss from pre-operative weight was 15.5%. Pathological and treatment characteristics are outlined in *Table 1*.

Plasma glucagon-like peptide-1

Although the shape of the post-prandial GLP-1 response curve was altered at the week 4 timepoint (P=0.01), overall suppression with treatment as determined by the area under the curve was not significant (P=0.08) (Figure 2). On post-hoc analysis, GLP-1 response at 30 minutes was lower after treatment (P<0.001). This is corroborated by a trend towards the suppression of early AUC (up to 60 minutes post meal ingestion, P=0.053), but no evidence of a significant difference in overall AUC (P=0.30). There was also a trend towards a reduction in peak post-prandial GLP-1 (median [IQR] – week 0: 145 [93 – 192] pM vs. week 4: 135 [72 - 152] pM, P=0.055). Post-mixed meal appetite and nausea VAS scores did not differ between visits (Supplementary Figure S4 (39)).

Plasma glucose and insulin

Post-prandial plasma glucose concentration curves were different at week 4, relative to pre-treatment (P=0.046), with a more sustained post-prandial glycemic response to 60 minutes post-prandially (week 0: 5.6 (1.4) mmol/L vs. week 4: 7.5 (2.1) mmol/L, P <0.001) (Figure 3A). Treatment was not associated with a reduction in fasting or peak post-prandial glucose concentrations (both P >0.99). There were no cases of biochemical reactive hypoglycemia before or after treatment, defined as a post-prandial glucose of <2.8mmol/L (41).

Post-prandial plasma insulin concentrations were not significantly different at week 4, compared with week 0 (P=0.69) (Figure 3B). However, there was a significant treatment x time effect, with a delayed peak insulin at week 4 (P <0.001), with the concentration at 30 minutes, on post-hoc analysis, approximately two-fold higher pre-treatment (mean (SD), week 0: 97.2 (51.8) vs. week 4: 44.0 (33.7) mU/L, P <0.001), and the concentration at 60 minutes almost four-fold higher post-treatment (week 0: 21.6 (11.2) vs. week 4: 79.5 (38.3) mU/L, P <0.001).

Body anthropometry and composition

There were no significant changes in body weight after four weeks of treatment with Octreotide LAR (Table 2). There was an increase in waist-hip ratio (WHR) after four weeks of treatment (mean (SD), week 0: 0.88 (0.1) vs. week 4: 0.90 (0.1), P=0.02), although no change in absolute or relative body fat mass, or in skeletal muscle mass was observed.

Eating behavior

Motivation to eat

No difference was observed in appetitive behavior, as measured by PRT breakpoint (the last completed ratio), after four weeks of clinical treatment (median [IQR], week 0: 960 [400 – 1280] vs. week 4: 640 [320 – 1120], P=0.41) (Figure 4). Absolute number of clicks did not differ before and after treatment (mean (SD), week 0: 1479 (707) vs. week 4: 1251 (716) clicks, P=0.52) (Figure 4). These results occurred in the context of analogous pre-PRT plasma GLP-1 concentrations (P=0.25) and VAS appetite ratings, the latter of which suggests no change in 'pleasure' to receive food associated with treatment (P=0.46). More significant plasma GLP-1 suppression with Octreotide LAR correlated significantly with an increase in eating motivation as measured by total clicks during the PRT (P=0.026, R^2 = 0.59) (Supplementary Figure S5 (39)).

Ad libitum food intake and macronutrient preference

There was no difference in total energy intake during the *ad libitum* buffet meal before and after treatment (mean (SD), week 0: 796 (280) kcal vs. week 4: 847 (229) kcal, P=0.46) (Figure 5A), nor any change in macronutrient selection (P=0.26) (Figure 5B).

Importantly, % fat intake was not related to relevant symptoms, such as diarrhea (P=0.70), loss of appetite (P=0.58), and Sigstad score (P=0.18), but was significantly associated with increased reflux-like symptoms (P=0.009, R^2=0.70).

Functional MRI tasks

In the food picture evaluation fMRI task, functional region of interest (fROI) analysis using 2-factor repeated-measures ANOVA, including ROI and treatment visit as within subject factors, there were no significant ROI x visit interactions for the BOLD signal for any of the following contrasts: high-energy food vs. low-energy food [F(2.4, 16.4) = 0.713, P=0.525], high- or low-energy food vs. object [F(2.8, 19.3) = 1.62, P=0.314], high-energy food vs. object [F(2.8, 19.5) = 2.07, P=0.14], and low-energy food vs. object [F(2.8, 19.6) = 0.411, P=0.73] (Figure 6A-D). There were also no significant main effects of visit for any of these picture contrasts, independent of ROI (see Figure 6 legend for statistics). Thus, treatment with Octreotide LAR had no significant effect of high-energy or low-energy food cue reactivity.

For food picture appeal rating (relative to objects), there was also no significant energy density x visit interaction [F(1,7) = 2.396, P=0.166] nor main effect of visit (independent of energy density) [effect size mean ± SEM [95% CI] -0.110 ± 0.152 [-0.470, 0.252], F(1,7) = 0.515, P=0.496] (Figure 6F).

Thus, treatment with Octreotide LAR had no effect on food appeal independent or dependent on energy density.

In whole-brain analysis for the food picture evaluation fMRI task, there were no clusters displaying significant differences in BOLD signal (voxel-wise false discovery rate (FDR) P<0.05; nor cluster-wise family wise error (FWE) Z>2.1, P<0.05) between visits for the high-energy > low-energy food, high-energy food > object, low-energy food > object, nor any food (high-energy or low-energy food) > object picture contrasts.

The control auditory-motor-visual (AMV) fMRI task was designed to account for non-specific BOLD signal changes between visits.(38) In fROI analysis, no significant differences in BOLD signal within any fROI was found comparing before and after treatment (ROI x visit interaction: P=0.44, main effect of visit: P=0.46, Figure 6E).

Potential confounding variables

There were no significant differences between visits in variables that could have confounded BOLD signal responses: pre-fMRI composite appetite VAS ratings (median (range), week 0: 6.6 (2.4 – 7.7) vs. week 4: 5.4 (2.7 – 7.9), *P*=0.64), pre-fMRI nausea and sleep VAS ratings (Supplementary Figure S4 (39)), BMI (*P*=0.21), % body fat mass (*P*=0.58), pre-scan energy intake (400 kcal at mixed meal given to all participants), head motion during food picture evaluation fMRI task [average relative motion (mm per volume), median (IQR) week 0: 0.09 (0.08, 0.15), week 4: 0.14 (0.08, 0.16), Wilcoxon signed rank test *P*=0.074), with no participants having motion > 0.28 mm per volume].

Post-prandial symptoms and health-related quality of life

There were no significant differences in reported HRQL outcomes before vs. after treatment with Octreotide LAR, with global HRQL remaining stable (P=0.94) (Table 3).

When comparing key appetite and nutrition-related patient-reported outcomes, Sigstad score was lower after treatment (median (range), week 0: 12 (2-28) vs. week 4: 8 (3-18), P=0.039) (Figure 7, Table 3). The degree of plasma GLP-1 suppression (delta AUC 0-150 mins) did not correlate with improvement in Sigstad score (P=0.58, R^2=0.05). However, EORTC symptom appetite loss severity scores did not change significantly with Octreotide LAR treatment: median (range) week 0: 66.7 (0 – 66.7), week 4: 0 (0 – 66.7) (P=0.063) (Table 3).

DISCUSSION

This pilot study shows that it is feasible to characterize the gut-brain axis and its relation to food reward and eating behavior in a cohort post esophagectomy. Notwithstanding, 10mg Octreotide LAR was not sufficient to change gut hormone response, body weight, appetitive behavior or *ad libitum* food intake.

Somatostatin analogues have been used in surgical contexts to reduce post-prandial symptoms and can have beneficial nutritional effects, including the restoration of post-operative weight loss (30, 42). There has, however, historically been no method to systematically analyze the factors underlying their potential beneficial effects in the context of major upper gastrointestinal resection. This limits the ability to appropriately target treatment to patients who are most likely to derive benefit. Recent developments in our understanding of how alterations in gut-brain axis signaling may be implicated in the pathophysiology of post-esophagectomy symptomatology and malnutrition (11), should enable a more systematic approach to therapeutic interventions. Moreover, while studies at least suggest a compelling rationale for long-term nutritional benefit with the use of Octreotide LAR, through mitigation of the exaggerated post-operative gut hormone response, this remains to be proven.

In this study, the first to assess Octreotide LAR in this context, the scientific thesis was that Octreotide administration, shown previously to acutely inhibit GLP-1 secretion and increase *ad libitum* food intake among patients post esophagectomy, would suppress gut hormones and potentially facilitate weight regain. In this model, GLP-1 was the preferred marker to represent general post-prandial gut hormone secretion. We anticipated that significant plasma GLP-1 suppression at the mixed meal challenge would also be associated with increased brain reward system food cue reactivity and eating behavioral changes. However, four weeks after a treatment dose of long-acting Octreotide, GLP-1 secretion, though blunted in the early post-prandial phase, was not sufficiently suppressed with the dosage used. Moreover, in addition to the absence of a change in brain reward

system food cue reactivity and eating behavior, there were no significant changes in body weight or muscle mass. Given the suboptimal plasma GLP-1 suppression, the observations described herein cannot be asserted to reflect observations occurring despite gut hormone suppression. This is important to bear in mind when interpreting these findings and could feasibly underlie the lack of a positive effect with regards to body weight and composition with treatment. This is particularly pertinent as the studies describing an impact of short-acting Octreotide did demonstrate significant GLP-1 attenuation (27, 28). There was no clear precedent on which to base the dosing in this clinical context. The 10mg dose was selected based on weighing the risks and potential benefits of treatment. In an already nutritionally vulnerable cohort, the ethical principle of non-maleficence guided us in choosing a dose less likely to elicit adverse effects. In a study of patients post gastrectomy, 10mg of Octreotide was sufficient to improve quality of life within four weeks and those requiring up-titration to 20mg were less likely to derive benefit overall (30). As such, in the clinical context, patients were commenced on a 10mg dose, and this trial of therapy continued for one month at which point a shared decision was made with the patient based on subjective and objective treatment benefit. Future longer-term studies, allowing dose titration to avoid side effects, will be required to determine whether a prolonged duration of treatment and/or higher doses of Octreotide may induce observable changes in eating behaviour and body composition.

Post-prandial hypoglycemia is a phenomenon that occurs after foregut surgery and may be related to exaggerated post-prandial gut hormone release (43, 44). In the current study, however, there were no cases of biochemical or symptomatic hypoglycemia post-prandially. Nonetheless, a minimally potent dose of a long-acting somatostatin analogue was associated with an increase in post-prandial plasma glucose concentrations and a corresponding alteration of the pattern of post-prandial plasma insulin dynamics, delaying its peak.

GLP-1 exerts its anorectic effects in the hypothalamic arcuate nucleus, but may also play a role in the

hedonic signaling pathways of food reward (12, 13, 17, 45). Indeed, in related surgeries, such as Roux-en-Y gastric bypass (RYGB) surgery, attenuation of the exaggerated post-prandial GLP-1 (and PYY) gut hormone secretion is associated with increased brain reward system food cue reactivity and food appeal (12). This study used an established fMRI paradigm to assess food cue reactivity (12, 36-38), assessing BOLD signal in *a priori* regions of interest within the brain reward system, to explore the relationship of medium-term Octreotide LAR therapy with anticipatory food reward, but did not find a significant increase, or even a trend for an increase, in BOLD signal to high-energy or low-energy foods to corroborate previous findings. However, the degree of plasma gut hormone suppression achieved was notably less, and as such these findings are not presented as an attempt to rebut previous work on this question. Future studies will need to explore the use of higher doses to ascertain the potential role for long-acting Octreotide in improving outcomes by influencing food reward signaling, as well as increasing the number of participants.

Eating behavior is the final step along the pathway assessed in this study. This comprised the PRT, exploring appetitive behavior, and an *ad libitum* buffet meal, to directly measure food intake and food selection. The latter is subject to external conditioned and/or unconditioned influences, such as post-prandial symptoms creating food avoidance, or social cues or contexts, respectively (21, 46). As noted, previous work in patients after esophagectomy reported a 1.5-fold increase in *ad libitum* energy intake and increased motivation to receive a sweet-fat stimulus in the fed state with acute Octreotide administration (27, 28), mirroring similar observations in RYGB cohorts (12). The findings outlined in this paper suggest there is an association between the degree of gut hormone suppression obtained and alterations in eating behavior, as those with greater plasma GLP-1 suppression were more likely to increase their motivation to receive a food reward. However, this finding did not translate into a subsequent increase in energy intake in these participants during the buffet meal.

The *ad libitum* buffet meal did not corroborate previous findings. However, along with the suboptimal

plasma GLP-1 suppression, the methodology previously utilized differed. This study utilized a real-world buffet style meal based on patient preferences, while previous work has used a method involving investigator-controlled and timed provision of a single food item until satiation was reached by the participant. This distinction introduces variables such as conditioned food avoidance, a phenomenon whereby learned behavior over time in an attempt to mitigate against post-ingestive symptoms can lead to active avoidance of certain foods or food quantities (46). Interestingly, and relevant to the complexity and myriad factors involved in eating behavior, at the baseline visit, energy intake was not significantly associated with the relative or absolute increase in plasma GLP-1 at the meal suggesting that participant's food ingestion was more strongly influenced by other factors besides satiety signaling.

Prior longitudinal work in an esophagectomy cohort has shown that the exaggerated enteroendocrine response corresponds with important appetitive symptoms, such as early satiety, and correlates with Sigstad score (11). In this study, treatment with Octreotide LAR was associated with a 30% relative reduction in Sigstad score, reflective of acute post-prandial symptom burden. Although limited by the subjective nature of questionnaire data, if this potential finding is validated in larger studies, this represents a promising option for the management of postprandial symptoms. Indeed, prolonged exposure to a reduced symptom profile may manifest in more profound positive changes in eating behavior and body weight over time.

Several limitations must be acknowledged, which should be viewed in the context of this as a pilot study. This was not a randomized or controlled study, thus findings – positive and negative – must be interpreted with caution, recognizing its inability to infer causality. Given the relatively small sample size, negative results must be interpreted with caution as a Type II error cannot be excluded. The unblinded uncontrolled design exposes these findings to the order effect, whereby participants may improve performance on repeat testing based on prior experience. Notwithstanding this, the gross

absence of differences in VAS scores between visits does indicate that the potential of bias at the buffet meal may be less likely. As noted, the degree of gut hormone suppression achieved with clinical treatment was less than expected, which also limits the interpretation of these findings. This factor precludes the representation of any observations herein as associated with significant attenuation of the post-prandial gut hormone response. We have discussed them instead in the context of Octreotide LAR therapy. However, this absence of evidence of suppression at the four-week timepoint does not serve as evidence of absence of suppression at some point earlier in the treatment period.

In conclusion, this pilot study outlines, in a post-esophagectomy cohort with significant weight loss, the parallel characterization of multiple levels of the gut-brain axis and eating behavior, which will benefit future work aiming to better elucidate the role of these processes in the etiology of post-operative malnutrition. Moreover, it provided insight into the impact of a 4 week 10mg Octreotide LAR treatment course on the postprandial gut hormone response, food reward, and eating behavior. Though gut hormone suppression was suboptimal, patients did not exhibit altered body weight or appetitive behavior. These findings may be used to inform the design of future studies investigating the value of gut hormone attenuation as a therapy for malnutrition and weight loss, while also improving our mechanistic understanding to facilitate the development of more targeted therapeutic strategies.

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Data Availability

The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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FIGURE LEGENDS

Figure 1. Study protocol flowchart for 'REWARD' study.

fMRI, functional magnetic resonance imaging; HR-QL, health-related quality of life; mGSRS, modified gastrointestinal rating scale; LAR, long-acting release. PRT, progressive ratio task.

Figure 2. Post-prandial plasma GLP-1 before and after treatment with octreotide LAR.

(A) Post-prandial plasma total GLP-1 secretion, (B) peak postprandial GLP-1, and (C) total post-prandial area under curve (AUC) from before to 150 minutes, and (D) early AUC of post-prandial GLP-1 secretion before and after treatment with Octreotide LAR (n=8). Panel A: The grey dotted line represents consumption of the standardized 400 kcal mixed meal. Repeated measures two-way ANOVA with post-hoc Bonferroni's multiple comparisons test: mean difference [95% confidence interval] 8.47 [-0.91, 17.85], F[1, 42] = 3.32, *P*=0.076, Cohen's d = -0.23. T=30, mean difference [95% CI] 53.37 [21.85, 84.89, *P*<0.001]. Panel B: Mean ± SEM, paired *t* test: mean difference [95% CI] -21.81 [-45.33, 1.70], t = 2.19, df = 7, *P*=0.064, Cohen's d = -0.44. Panel C: Mean ± SEM, paired *t* test: mean difference [95% CI] -44.61 [-90.09, 0.87], t = 2.32, df = 7, *P*=0.053, Cohen's d = -0.75. Panel D: Mean ± SEM, paired *t* test: mean difference [95% CI] -43.93 [-137.3, 49.4], t = 1.11, df = 7, *P*=0.30, Cohen's d = -0.42. Abbreviations: GLP-1, glucagon-like peptide-1.

Figure 3. Post-prandial plasma glucose and insulin before and after treatment with octreotide LAR.

Time course of plasma (A) glucose and (B) insulin before and after standardized 400 kcal mixed meal with grey dotted line representing time of meal consumption (n=8). (A) Post-prandial plasma glucose concentrations were higher after treatment with octreotide LAR. 2-way repeated measures ANOVA with Bonferroni's multiple comparisons test: mean difference [95% confidence interval] -0.385 [-

0.763, -0.007], F[1, 42] = 4.23, *P*=0.046, Cohen's d = 0.26. (B) There was a treatment x time effect on post-prandial plasma insulin after treatment with a delayed peak, but no overall difference in post-prandial concentrations after treatment: mean difference [95% CI] -1.454 [-8.791, 5.883], F[1, 42] = 0.16, *P*=0.69, Cohen's d = 0.04. At T=30, post-treatment insulin concentration was significantly lower (mean difference [95% CI]: 53.29 [28.63, 77.95], *P* <0.001), while at T=60 insulin was significantly higher after treatment (mean difference [95% CI]: -57.89 [-82.55 to -33.23], *P* <0.001).

Figure 4. Progressive ratio task before and after treatment with octreotide LAR.

(A) Breakpoint and (B) total clicks in progressive ratio task (PRT) before and after treatment with octreotide LAR. Data given as median ± IQR (interquartile range), n=8. (A) Wilcoxon matched pairs signed rank test: *P*=0.41, Cohen's d = -0.47, (B) mean ± SEM, paired t test: mean difference [95% CI] -228 [-1030, 575.2], t = 0.67, df = 7, *P*=0.52, Cohen's d = -0.32.

Figure 5. *Ad libitum* buffet meal before and after treatment with octreotide LAR.

Octreotide LAR treatment was not associated with (A) a change in total energy intake at *ad libitum* buffet meal (mean ± SEM, paired t test: mean difference [95% CI] 50.65 [-101.0, 202.3], t = 0.79, df = 7, *P*=0.46), nor (B) macronutrient selection at *ad libitum* buffet meal 2-way (repeated measures ANOVA with post-hoc Bonferroni's multiple comparisons test: mean difference [95% confidence interval] -0.095 [-1.604, 1.414], F[1, 28] = 0.02, *P*=0.89, Cohen's d < 0.01); n=8. Abbreviations: CHO, complex carbohydrates.

Figure 6. Food picture evaluation and auditory-motor-visual fMRI tasks before and after treatment with octreotide LAR.

There were no significant differences in the magnitude of BOLD signal (%) in *a priori* regions of interest ROI) in the (A-D) food picture evaluation functional MRI task (amygdala, anterior insula, orbitofrontal cortex (OFC), putamen, nucleus accumbens, and caudate) before vs. after treatment for contrasts: (A) high-energy vs. low-energy food, mean difference [95% confidence interval] 0.038 [-0.096 – 0.173], F[1, 7] = 0.46, *P*=0.52, Cohen's d = -0.55, (B) high-energy or low-energy foods (vs. objects), mean difference [95% CI] 0.079 [-0.032, 0.191], F[1, 7] = 2.82, *P*=0.14, Cohen's d = -1.65, (C) high-energy foods (vs. objects), mean difference [95% CI] 0.097 [-0.007, 0.200], F[1, 7] = 4.88, *P*=0.06, Cohen's d = -1.54, and (D) low-energy foods (vs. objects), mean difference [95% CI] 0.062 [-0.094, 0.218], F[1, 7] = 0.89, *P*=0.38, Cohen's d = -1.14, nor (E) auditory-motor-visual control functional MRI task (bilateral superior temporal gyrus in auditory task, left precentral gyrus in motor task, and bilateral lingual gyrus in visual task), nor (F) appeal rating of low-energy or high-energy foods (vs. objects) in food picture evaluation functional MRI task. Analyses used 2-way repeated measures ANOVA including (A-E) visit and ROI, or (F) visit and energy density, as within subject factors with post-hoc Fisher least-significant difference test (n=8). Abbreviations: Amy, amygdala; AMV, auditory-motor-visual; BOLD, blood oxygen level-dependent; Caud, caudate; ED, energy density; HE, high-energy; Ins, anterior insula; LE, low-energy; Lingual, lingual gyrus; LpreCG, left precentral gyrus; NAcc, nucleus accumbens; OFC, anterior orbitofrontal cortex; Put, putamen; STG, superior temporal gyrus posterior division.

Figure 7. Sigstad dumping syndrome score before and after treatment with octreotide LAR.

Sigstad score was significantly lower after 4 weeks of treatment. Median ± interquartile range, Wilcoxon matched-pair signed rank test: *P*=0.039, Cohen's d = -0.61, n=8.

Table 1: Clinicopathological characteristics of the study cohort

	Cohort N = 8
Clinical Characteristics	
Age, mean (SD)	62.8 (9.4)
Sex, n (%)	
Female	1 (12.5)
Male	7 (87.5)
Body weight at 1st study visit, kg, mean (SD)	68.6 (12.8)
BMI at 1st study visit, kg/m^2, mean (SD)	22.4 (2.8)
Pre-illness BMI, kg/m^2, mean (SD)	27.2 (3.6)
Preoperative BMI, kg/m^2, mean (SD)	26.5 (3.0)
Post-diagnosis body weight loss (%), mean (SD)	17.5 (6.1)
Post-operative body weight loss (%), mean (SD)	15.5 (5.8)
Time since operation, months, median (range)	23.6 (7.3)
Pathology	
Adenocarcinoma, n (%)	6 (75.0)
SCC, n (%)	2 (25.0)
Operation, n (%)	
2-stage oesophagectomy	3 (37.5)
Transhiatal oesophagectomy	4 (50.0)
3-stage oesophagectomy	1 (12.5)
Neoadjuvant therapy, n (%)	**2 (25.0)**
Chemoradiotherapy	1 (50.0)
Chemotherapy	1 (50.0)

Abbreviations: BMI, body mass index (kg m^{-2}); SCC, squamous cell carcinoma; SD, standard deviation.

Table 2. Body weight, composition, and anthropometry in patients before and after treatment with long-acting Octreotide.

	Week 0 N = 8	Week 4 N = 8	***P*-value**
Anthropometry			
Body weight, kg, mean (SD)	68.6 (12.8)	69.2 (13.4)	0.129
BMI, kg/m^2, mean (SD)	22.4 (2.6)	22.7 (3.0)	0.207
% Body weight change, mean (SD)	-	0.68 (1.3)	0.185*
Mid-upper arm circumference, cm, mean (SD)	25.6 (1.4)	25.8 (2.4)	0.785
Waist:Hip Ratio, mean (SD)	0.88 (0.1)	0.90 (0.1)	**0.024**
Body Composition			
Skeletal muscle mass, kg, mean (SD)	25.4 (5.8)	25.5 (6.1)	0.914
Fat-free mass, kg, mean (SD)	52.7 (9.8)	52.6 (10.2)	0.965
Fat mass, kg, mean (SD)	16.0 (4.9)	16.6 (5.0)	0.406
Fat mass, %, mean (SD)	23.3 (5.2)	23.8 (5.3)	0.582

*Paired t test unless otherwise specified. *one-sample t test. Abbreviations: BMI, body mass index; SD, standard deviation.*

Table 3. Selected health-related quality of life and nutrition-specific symptoms before and after treatment with Octreotide LAR

	Week 0 N = 8	**Week 4** N = 8	**Mean difference (SD)**	***P*-value**
Quality of life outcomes, EORTC, median (range)				
Health-related quality of life	62.5 (50.0 – 91.7)	66.7 (41.7 – 83.3)	1.0 (10.4)	0.938
Role function	91.7 (33.3 – 100.0)	100.0 (33.3 – 100.0)	6.3 (25.1)	0.625
Physical function	90.0 (66.7 – 100.0)	86.7 (46.7 – 100.0)	-3.3 (8.7)	0.719
Social function	66.7 (16.7 – 100.0)	66.7 (33.3 – 100.0)	-1.0 (11.3)	0.750
Cognitive Function	83.3 (50.0 – 100.0)	66.7 (50.0 – 100.0)	-4.2 (11.8)	0.500
Dumping syndrome				
Sigstad Score, median (range)	**12 (2 – 28)**	**8 (3 – 18)**	**-3.9 (3.7)**	**0.039**
Clinically significant Sigstad score >7, n (%)	7 (87.5)	4 (50.0)	-	0.282[#]
Select EORTC symptoms, median (range)				
Appetite loss	66.7 (0.0 – 66.7)	0.0 (0.0 – 66.7)	-33.3 (30.9)	0.063
Early satiety	66.7 (0.0 – 66.7)	33.3 (0.0 – 100.0)	-8.3 (38.8)	0.359
Eating difficulties	29.2 (8.3 – 41.7)	33.3 (8.3 – 50.0)	3.1 (13.3)	0.813
Nausea	16.7 (0.0 – 33.3)	16.7 (0.0 – 33.3)	<0.1 (8.9)	0.563
Diarrhea	0.0 (0.0 – 33.3)	33.3 (0.0 – 66.7)	14.3 (17.8)	0.250
Reflux (acid / bile)	33.3 (0.0 – 50.0)	16.7 (0.0 – 83.3)	<0.1 (17.8)	0.938
Dysphagia	0.0 (0.0 – 11.1)	0.0 (0.0 – 11.1)	<0.1 (4.7)	0.500

All analyses used Wilcoxon matched-pairs signed rank tests, unless otherwise specified: [#]Fisher's

exact test. For quality of life outcomes, possible scores range from 0 – 100, with 100 representing an optimal score. Abbreviations: EORTC, European Organization for Research and Treatment of Cancer; SD, standard deviation.

Figure 1

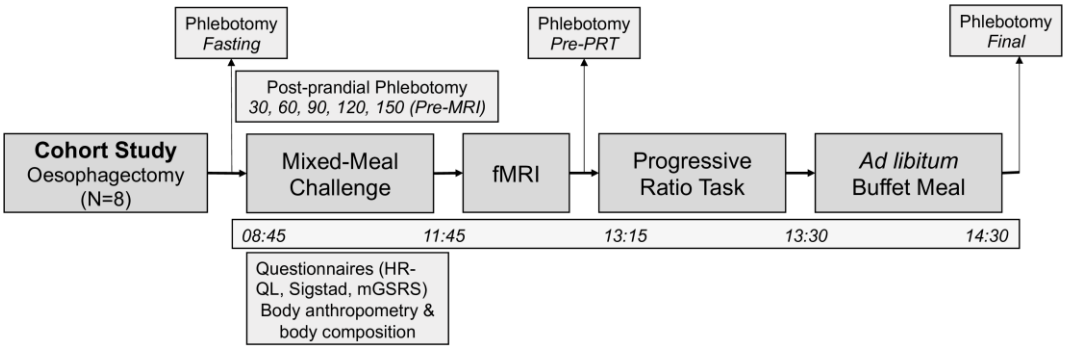


Figure 2

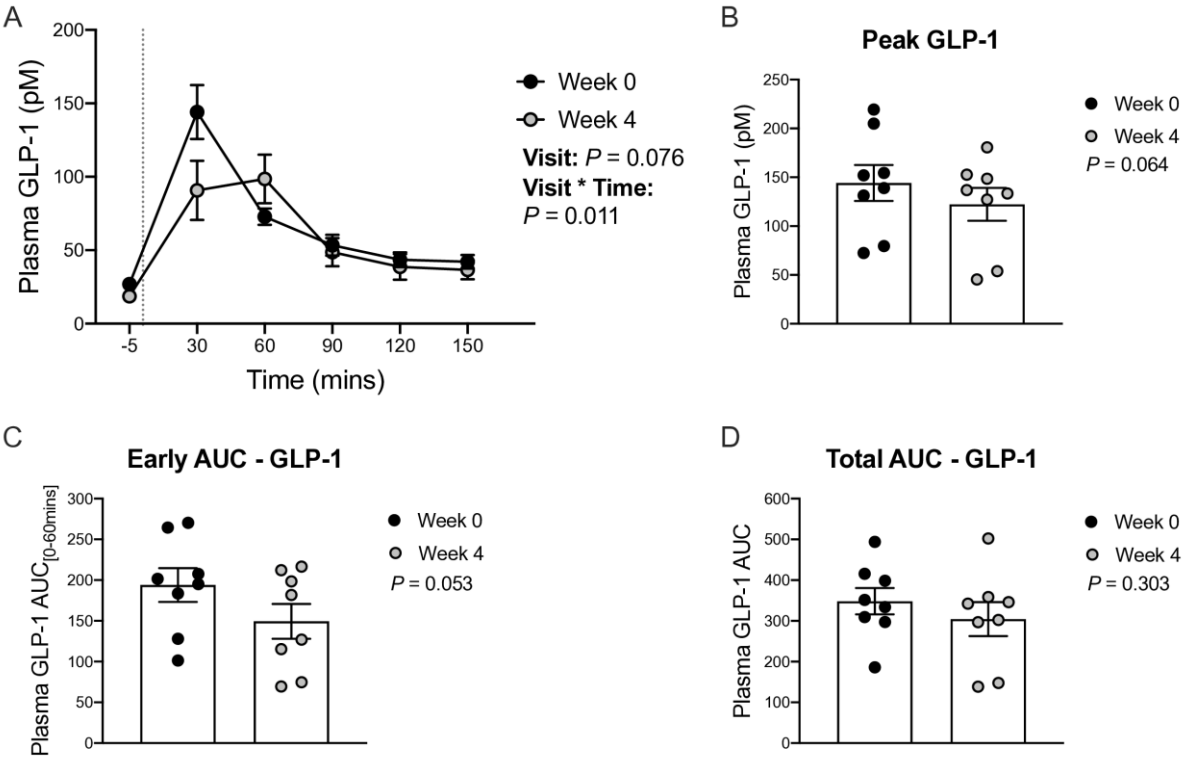
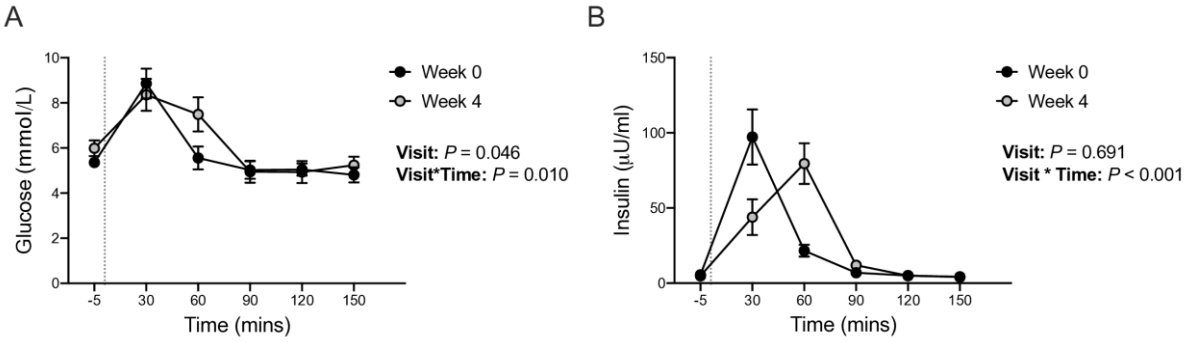


Figure 3



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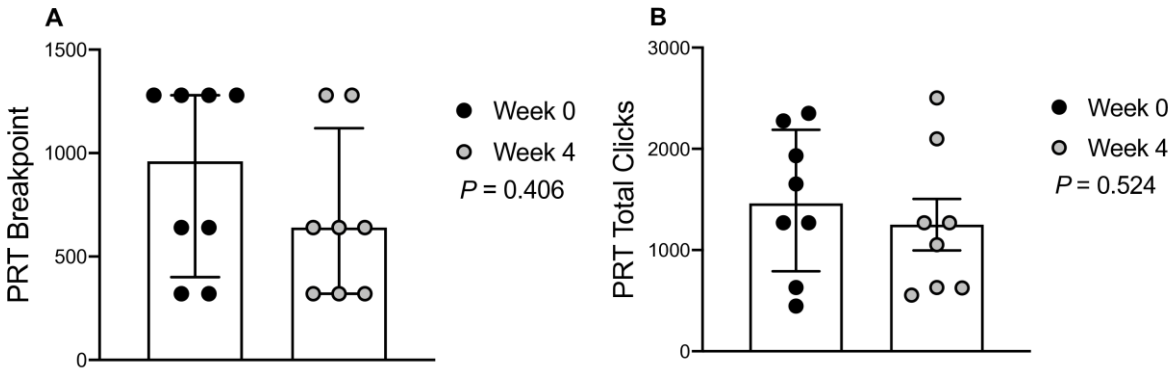

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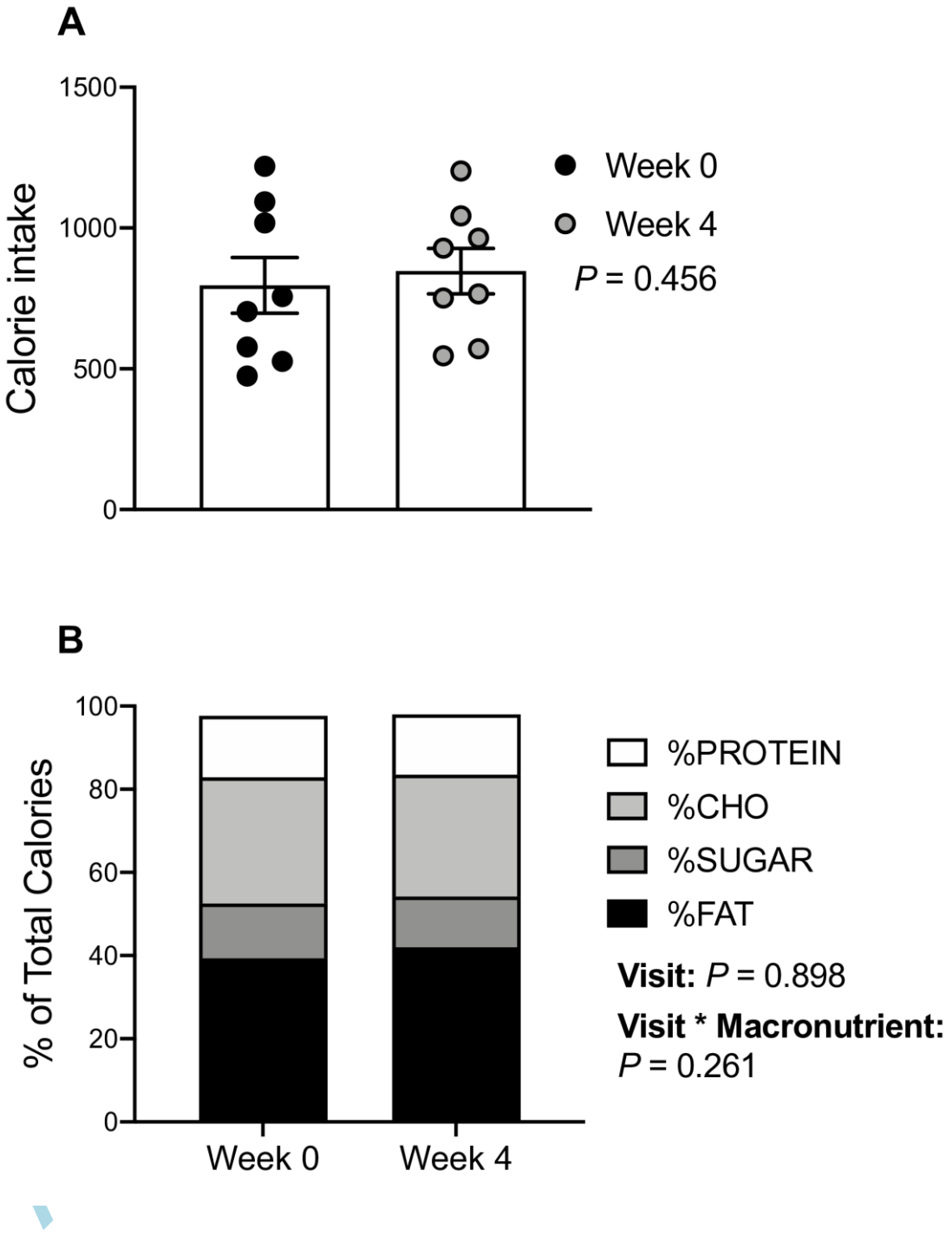

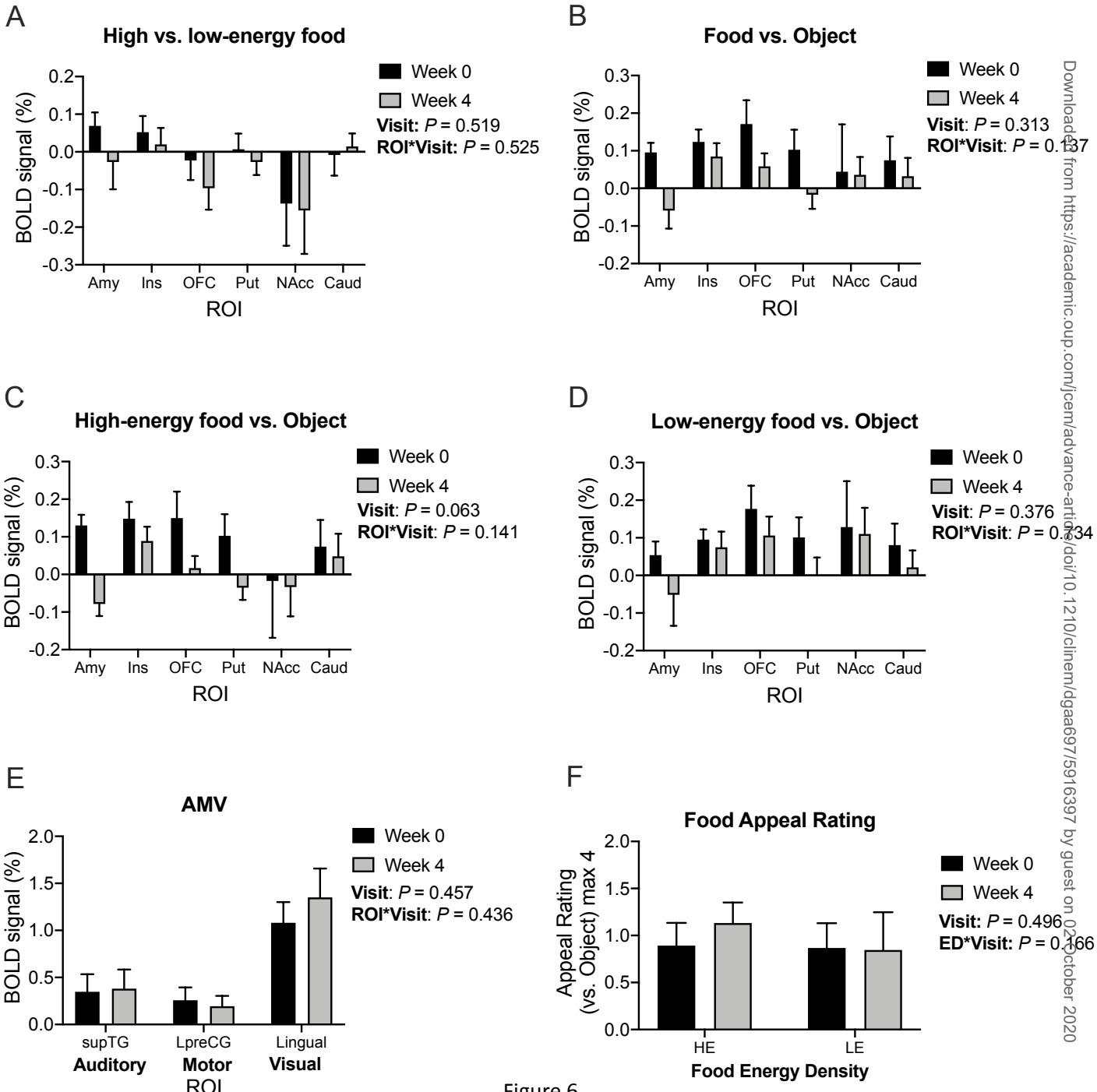

Figure 6

Figure 7