

An extract of hops (*Humulus lupulus* L.) modulates gut peptide hormone secretion and reduces energy intake in healthy-weight men: a randomized, crossover clinical trial

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ABSTRACT

Background: Gastrointestinal enteroendocrine cells express chemosensory bitter taste receptors that may play an important role in regulating energy intake (EI) and gut function.

Objectives: To determine the effect of a bitter hop extract (*Humulus lupulus* L.) on acute EI, appetite, and hormonal responses.

Methods: Nineteen healthy-weight men completed a randomized 3-treatment, double-blind, crossover study with a 1-wk washout between treatments. Treatments comprised either placebo or 500 mg of hop extract administered in delayed-release capsules (duodenal) at 11:00 h or quick-release capsules (gastric) at 11:30 h. Ad libitum EI was recorded at the lunch (12:00 h) and afternoon snack (14:00 h), with blood samples taken and subjective ratings of appetite, gastrointestinal (GI) discomfort, vitality, meal palatability, and mood assessed throughout the day.

Results: Total ad libitum EI was reduced following both the gastric (4473 kJ; 95% CI: 3811, 5134; $P = 0.006$) and duodenal (4439 kJ; 95% CI: 3777, 5102; $P = 0.004$) hop treatments compared with the placebo (5383 kJ; 95% CI: 4722, 6045). Gastric and duodenal treatments stimulated pre-lunch ghrelin secretion and postprandial cholecystokinin, glucagon-like peptide 1, and peptide YY responses compared with placebo. In contrast, postprandial insulin, glucose-dependent insulinotropic peptide, and pancreatic polypeptide responses were reduced in gastric and duodenal treatments without affecting glycemia. In addition, gastric and duodenal treatments produced small but significant increases in subjective measures of GI discomfort (e.g., nausea, bloating, abdominal discomfort) with mild to severe adverse GI symptoms reported in the gastric treatment only. However, no significant treatment effects were observed for any subjective measures of appetite or meal palatability.

Conclusions: Both gastric and duodenal delivery of a hop extract modulates the release of hormones involved in appetite and glycemic regulation, providing a potential “bitter brake” on EI in healthy-weight men. *Am J Clin Nutr* 2022;115:925–940.

Keywords: hops, appetite, energy intake, ghrelin, cholecystokinin, glucagon-like peptide 1, peptide YY, pancreatic polypeptide, insulin, glucose-dependent insulinotropic peptide

Introduction

Control of energy intake (EI) is central to the success of interventions designed to manage body weight (1) and the consequences of obesity (2–6). The gastrointestinal (GI) tract expresses an array of chemosensory receptors and transporters that provide critical inputs into the acute regulation of energy intake, detecting and relaying to the brain the location, chemical composition, and concentration of nutritive and nonnutritive compounds in the gut (7, 8). Obesity and poor weight-loss outcomes are associated with impaired gut–brain axis signaling (9–13), which may contribute to overeating and poor adherence to dietary restriction (14–17). Approaches that restore or enhance gut–brain axis signaling may address this underlying feedback dysregulation. Indeed, enhancement of gut–brain axis signaling may explain many of the benefits of gastric bypass surgery (18),

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Supplemental Tables 1–5 and Supplemental Figures 1–5 are available from the “Supplementary data” link in the online posting of the article and from the same link in the online table of contents at <https://academic.oup.com/ajcn/>.

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Abbreviations used: CCK, cholecystokinin; CO₂, carbon dioxide; EEC, enteroendocrine cell; EI, energy intake; GI, gastrointestinal; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; HbA1c, glycated hemoglobin; hT2R, human bitter taste receptor; LSD, least significant difference; POMS, Profile of Mood States; PP, pancreatic polypeptide; PYY, peptide YY; T2R, bitter taste receptor; VAS, visual analog scale.

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dietary strategies (e.g., high fiber/protein), and pharmaceutical interventions (19, 20) on the control of EI. Importantly, GI chemosensory mechanisms are readily accessible to dietary manipulation and represent an unexploited source of weight management targets (21–23).

Bitter taste receptors (T2Rs) comprise a family of 25 G protein–coupled receptors that are expressed in multiple tissues, including enteroendocrine cells (EECs), of the GI tract (24–26) and are thought to have evolved a chemosensory role in the detection of potential harmful substances, limiting their ingestion and absorption (27, 28). In vitro, T2R agonists stimulate the release of peptide hormones, such as ghrelin, cholecystokinin (CCK), and glucagon-like peptide 1 (GLP-1), from gut enteroendocrine cells (29–32). These gut peptide hormones play a key role the homeostatic regulation of appetite, energy intake, gut function, hedonic food perceptions, and nutrient metabolism (33–37). A number of clinical studies using either encapsulation or intragastric and intraduodenal infusion of bitter tastants have demonstrated effects ranging from increased gut peptide secretion, reduced energy intake or rate of gastric emptying, modifications in subjective ratings of hunger and fullness, and altered glycemic regulation (38–44), although these anorexigenic effects are inconsistent (43, 45–47), necessitating further investigation of this response.

Hops (*Humulus lupulus* L.) contain a range of bitter compounds, including α -acids (humulone, adhumulone, and cohumulone) and β -acids (lupulone, adlupulone, and colupulone) that are known ligands for human bitter taste receptors (48). They have a long history of use as food additives and bittering agents in brewing, as well as in traditional medicine, and have been shown in vitro to stimulate Ca^{2+} -dependent CCK release from EEC cells (32). Administration of hop-derived extracts has also been shown to reduce body weight and fat mass and improve glucose homeostasis in both rodent (32, 49–56) and human studies (39, 51, 57). In addition, our laboratory has demonstrated that administration of a supercritical carbon dioxide (CO_2) hop extract can reduce subjective ratings of hunger during water-only fasting (58).

Here we investigate the efficacy and GI site of action of a bitter supercritical CO_2 hop extract to modify acute energy intake, hormonal and glycemic responses, and subjective ratings of appetite, GI discomfort, meal palatability, and mood in healthy-weight men.

Methods

Participants

Healthy-weight men (aged 18–55 y), with a BMI (in kg/m^2) between 20 and 25 were recruited by advertisement in the Auckland region, New Zealand. A telephone prescreening interview to determine eligibility of interested individuals was followed by a screening visit to verify eligibility by measurement of height and weight, assessment of oral bitter taste sensitivity to the hops extract, and determination of health status by self-report and blood tests [glycated hemoglobin (HbA1c), liver function, full blood count, iron status].

Participants were excluded if they had a diagnosed medical condition or were on medications known to affect taste, appetite-related parameters, metabolism, or GI function. Exclusions also

applied to participants currently on a weight-loss program or taking weight-loss medication or who had significant weight loss or gain (>5 kg) within the past 6 mo, were smokers, or had a history of alcohol or drug abuse. Participants with hypersensitivities or allergies to any foods or ingredients included in the study, as well as those who disliked or were unwilling to consume items listed as study foods, were unwilling or unable to comply with the study protocol, or were participating in another clinical intervention trial, were also excluded.

All participants provided informed consent prior to clinical trial enrollment. Human ethics approval was obtained from the Northern B Health and Disability Ethics committee (ref. 14/NTB/25) and the trial registered at the Australian and New Zealand clinical trials registry (ref. ACTRN12614000434695). The study was conducted at the Consumer and Products Insights facility of the New Zealand Institute for Plant and Food Research Limited (Auckland, New Zealand) from March to June 2014.

Study design

A randomized, double-blind, placebo-controlled, 3-treatment crossover study design was used with 3 treatment arms (**Supplemental Table 1**). These were hop extract (500 mg) targeted for release into the stomach (gastric), hop extract (500 mg) targeted for release in the proximal small intestine (duodenum), and a vehicle control (placebo). Randomization was conducted using a 3×3 Latin square balanced for treatment order and carryover effects (59, 60). Blinding of treatments was performed by an independent individual unaware of the treatment allocation.

Three 1-d visits were required with a washout period of at least 1 wk between visits. The daily protocol is shown in **Figure 1**. Food intake and subjective measures of appetite, GI discomfort, vitality, meal palatability, and mood were assessed during fully supervised study days.

The primary outcome was ad libitum energy intake at the test meals. Secondary outcomes were blood measurements and subjective ratings of appetite, thirst, GI discomfort, vitality, and meal palatability, whereas mood state was an exploratory outcome.

Treatments

To maintain treatment blinding, all treatments contained 2 sets of opaque capsules. One set, administered at 11:00 h, included delayed-release capsules (DRCaps, size 0; Capsugel) designed to release their contents ~ 50 –70 min after ingestion, increasing the likelihood of delivery to the duodenum (61). The second set, given at 11:30 h, included standard hydroxypropylmethylcellulose capsules (Vcaps, size 0; Capsugel) designed to release rapidly in the stomach. The timing of capsule administration was chosen so that the treatment capsules would probably have released their contents in the stomach or duodenum before the ad libitum lunch (12:00 h).

The 3 treatment groups were as follows: the placebo group included 2 vehicle control delayed-release capsules (11:00 h) followed by 2 vehicle control standard-release capsules (11:30 h), the gastric group had 2 vehicle control delayed-release capsules (11:00 h) followed by 2 standard-release capsules (11:30 h) containing hop extract; and the duodenum group had

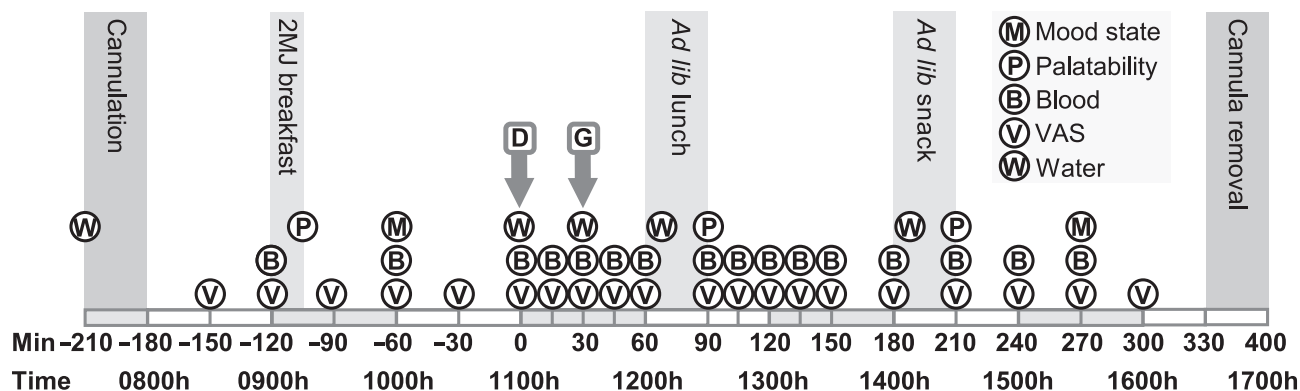


FIGURE 1 Protocol for study visits 1–3. Participants arrived fasted (07:30 h) and were cannulated and provided with a fixed-energy (2 MJ) breakfast (09:00 h) that they had to complete. Treatments along with matched placebo capsules targeting the duodenum (D) or gastric (G) compartments were administered at 11:00 h (T = 0 min) and 11:30 h (T = 30 min), respectively. Participants were provided with ad libitum lunch (12:00 h) and snack (14:00 h) outcome meals and directed to eat until comfortably full. The amount and timing of fluid intake were controlled during study visits with 150 mL of water (W) required to be consumed prior to cannulation and 250 mL with each treatment administration and test meal. Blood samples (B) and VAS ratings (V) of appetite, thirst, vitality, and gastrointestinal discomfort related measures were collected throughout the day. Ratings of meal palatability (P) were assessed using VAS scales immediately after every meal. Mood state (M) was assessed in the morning and afternoon using the Profile of Mood State questionnaire. *Ad lib*, ad libitum; VAS, visual analog scale.

2 delayed-release capsules containing hop extract (11:00 h) followed by two vehicle control standard-release capsules (11:30 h).

Each hop treatment capsule contained a proprietary formulation (Amarasate; Plant & Food Research Ltd) comprising 250 mg of a food-safe, supercritical CO₂ extract of hop cones (*H. lupulus* L. “Pacific Gem”; New Zealand Hops Ltd), mixed with 125 mg of canola oil as an excipient (a 2:1 hops/oil ratio). The vehicle control capsules used in the placebo treatment and for blinding in the gastric and duodenal treatments contained 125 mg of canola oil. All capsules were filled in-house using the Capsugel Profiller system (Capsugel) with a coefficient of variation of 2% for loading accuracy.

The α - and β -acid composition of the hop supercritical CO₂ extract comprised 51.5% total α -acids (cohumulone, 21.1%; humulone, 22.3%; and adhumulone, 8.2%) and 28.3% total β -acids (colupulone, 19.7%; lupulone, 6.0%; and adlupulone, 3.1%), as determined by HPLC (**Supplemental Figure 1**) with reference to the American Brewing Association ICE-3 standard as described in the European Brewery Convention for HPLC analysis of hop α - and β -acids (62). The α - and β -acid composition of the hop formulation has previously been shown (58) to remain stable over the duration of use in the current study.

Study visits 1–3

Participants arrived at the study facility by 07:30 h on test days in an overnight fasted state (no food or drink apart from water since 22:00 h), having abstained from excessive exercise or alcohol consumption the day before. An indwelling venous cannula was inserted into a forearm vein for repeated blood collection. **Figure 1** shows the study visit protocol, including timing of meals, treatment administration, and the collection of blood and behavioral measures. During free time between the meals and questionnaires, participants remained inside the

facility but were free to read, watch TV, or access the Internet on their own devices. Participants were free to leave the facility after completion of the final study questionnaire and removal of the cannula at 16:00 h.

Fixed-energy breakfast, ad libitum meals, and EI

The fixed-energy (2 MJ) breakfast (**Supplemental Table 2**) consisted of puffed rice cereal with low-fat milk and white bread with margarine and jam. Participants were instructed to consume the entire breakfast within 15 min (verified by visual inspection). The outcome ad libitum lunch [12:00 h, time (T) = 60 min] was a savory buffet restricted to a beef and tomato pasta sauce and boiled pasta spirals provided in separate containers. Participants were instructed to apportion the pasta and sauce at their preferred ratio into a separate bowl for eating, refilling as required. Ham sandwiches, cut into quarters with the crusts removed, were provided for the outcome afternoon snack (14:00 h, T = 180 min). Both ad libitum meals were provided in excess, with participants instructed that they had 30 min to eat until they were comfortably full. To minimize distractions, all meals were provided in individual booths with participants instructed not to talk, read, or use mobile phones or electronic devices and to remain in the booth for the designated time. Meals were weighed by 2 separate observers before and after consumption, and energy, fat, carbohydrate, and protein intakes were calculated with the use of the dietary software program FoodWorks (Professional Edition, version 5; Xyris Software). All meals were designed to have low phytochemical content to minimize nonspecific effects on appetite (63).

The amount and timing of fluid intake were also controlled with 150 mL of water only required to be consumed prior to cannulation (07:30 h) and 250 mL with each treatment administration (11:00 h and 11:30 h) and during each test meal (12:00 h and 14:00 h).

Behavioral measures

Visual analog scales (VASs) were used to assess subjective feelings of hunger, fullness, satiety, and prospective consumption following the methodology outlined in Flint et al. (64) and Blundell et al. (65). Additional VASs were used to assess thirst; measures of vitality (energy levels and relaxation); GI discomfort, including nausea, urge to vomit, bloating, abdominal discomfort, and heartburn [adapted from Bovenschen et al. (66)]; and meal palatability (64) (pleasantness, visual appeal, smell, taste, aftertaste, and overall palatability). The VAS questions and anchor statements are provided in **Supplemental Table 3**. Participants marked their responses by placing a vertical line across the 100-mm scale according to subjective feelings, with responses recorded to the nearest millimeter.

Changes in mood states were assessed at 10:00 h ($T = -60$) and 15:30 h ($T = 270$) using the original version of the Profile of Mood States (POMS) questionnaire (67), a 65-item inventory of 6 subscales: tension–anxiety, depression–dejection, anger–hostility, vigor–activity, fatigue–inertia, and confusion–bewilderment. Participants rated “How are you feeling right now” for each mood descriptor on a 5-point scale anchored by 0 (*not at all*) and 4 (*extremely*). The total mood disturbance score was computed by adding the 5 negative subscale scores (tension, depression, anger, fatigue, confusion) and subtracting the vigor score.

The occurrence of adverse events was recorded for each study visit with participants describing symptoms and their severity using a 3-point scale of mild, moderate, or severe. Participants were also asked to recall any delayed symptoms/events during the washout period at their next visit.

Blood measurements

Blood for peptide hormones analysis was collected into prechilled 5-mL EDTA tubes (BD Vacutainer; BD) containing a dipeptidyl-aminopeptidase IV inhibitor (25 μ L of a 2-mM solution of Diprotin A; Peptides International) and a general protease inhibitor cocktail (Complete Mini EDTA-free protease inhibitor; Roche) (182 μ L of solution made up of 1 tablet in 2 mL of water). Blood for plasma glucose analysis was collected into sodium fluoride/potassium oxalate Vacutainer tubes (BD). Upon collection, samples were immediately centrifuged ($1500 \times g$ for 10 min at 4°C) and the plasma snap frozen on dry ice before storage at -80°C until analysis.

Ghrelin (active), GLP-1 (active), peptide YY (PYY) (total), insulin, glucose-dependent insulintropic polypeptide (GIP) (total), and pancreatic polypeptide (PP) concentrations were measured using a multiplexed magnetic bead assay (HMHMAG-34 K; Merck-Millipore). Samples were assayed in duplicate and plates read using a Magpix system (Luminex) with concentrations determined using a 5-parameter curve fit in Analyst 5.1 (Miliplex). Plasma CCK concentrations were determined in duplicate by radioimmunoassay (EURIA-CCK; Eurodiagnostica) as per the manufacturer’s instructions, with CCK standards formulated in pooled charcoal-stripped human plasma. Assay quality control data are given in **Supplementary Table 4**. Plasma glucose was analyzed by Lab Services (North Shore Hospital Lab Services) using the hexokinase method on a Dimension Vista 1500 (Siemens AG).

Statistical analysis

The study sample size was based on a previously reported preload appetite study in men (68). A sample size of 17 participants was calculated to detect a 500-kJ difference in EI based on a standard deviation of 686 kJ, 80% power, and α level of 0.05 (69). An additional 3 participants were added to allow for dropouts.

A per-protocol analysis was used for participants with $>95\%$ of data obtained, and only available data were included in the analyses. Time profile data including VAS ratings and blood biomarkers were analyzed with the use of a linear mixed model (SAS software, PROC GLIMMIX function, version 9.4; SAS Institute) with subject by time point included as a random effect and treatment, time point, visit number, and treatment order (1 of 6 possible treatment sequences allocated to each subject) and their respective interactions included as fixed effects. Where there was evidence of a treatment effect, either by a statistically significant treatment or treatment \times time interaction ($P < 0.05$), F tests for treatment differences at each time point were conducted using the “sliceby” command to give an indication of when these effects were being experienced. Where these were significant ($P < 0.05$), Fisher’s protected least significant difference (LSD) post hoc analysis was used for pairwise comparisons between the 3 treatments.

Total AUC data were calculated from time 0 to 270 min for blood biomarkers and from 0 to 300 min for VAS measures by numerical integration (Simpson’s rule) using the Bolstad package (70) in R (71). AUC data were analyzed using a linear mixed model (SAS 9.4) with treatment, visit number, and treatment order as fixed effects and subject as a random effect. Where there was evidence of a main treatment effect ($P < 0.05$), Fisher’s protected LSD post hoc analysis was used for pairwise comparisons between the 3 treatments. EI data were analyzed in the same way, with models fitted separately for the snack, lunch, and total kJ intake measures. For meal palatability measures, an additional fixed factor of meal (breakfast, lunch, and snack) was included in the linear mixed model, whereas analysis of POMS subscales included the additional fixed factor of time (pre/post). In all these cases, subject was included as a random effect. Residual plots were inspected to confirm that normality and constant variance assumptions were met. Where appropriate, data were log-transformed prior to analysis with results presented as back-transformed values.

Spearman rank correlations were performed to establish if subjective ratings of GI discomfort ($\text{AUC}_{0-300 \text{ min}}$) correlated with total EI using GraphPad Prism version 6.07 for Windows (GraphPad Software).

Statistical significance was assessed at $P < 0.05$ and results presented as means with 95% CI or \pm SEM as indicated.

Results

Participants

Of the 20 healthy-weight male participants randomly assigned into the trial, 19 completed all 3 arms of the study, with 1 participant excluded for failure to comply with study protocol (see Consolidated Standards of Reporting Trials flow diagram, **Supplemental Figure 2**). Characteristics of the 19 participants

TABLE 1 Characteristics of the 19 male participants who completed all 3 treatment arms¹

| Characteristic | Value |
|---------------------------|-------------------------|
| Age, y | 28.9 ± 10.4 (18–54) |
| Height, m | 1.80 ± 0.08 (1.66–1.95) |
| Body weight, kg | 76.1 ± 8.3 (60.4–94.5) |
| BMI, kg/m ² | 23.5 ± 1.4 (20.9–25.0) |
| Ethnicity, ² n | |
| New Zealand European | 13 |
| Māori/Pacifica | 2 |
| Asian | 3 |
| Other | 1 |

¹Values are presented as mean ± SEM (range) unless otherwise indicated. All measurements were recorded at the screening visit.

²Ethnicity was assessed by self-report.

included in the final analysis of energy intake and subjective behavioral measures are shown in **Table 1**. A second participant was excluded from blood sample analysis only because of repeated cannula failures and inability to obtain sufficient blood volume. Hence, data on blood biomarkers are presented for 18 participants.

EI at ad libitum meals

The effects of treatment on EI at the outcome ad libitum lunch and snack meals are shown in **Figure 2**. Total EI from the 2 outcome meals showed a highly significant effect of treatment ($F_{2,34} = 6.0$, $P = 0.006$), with significant reductions in EI for both the gastric (4473 kJ; 95% CI: 3811, 5134; $P = 0.006$) and duodenal (4439 kJ; 95% CI: 3777, 5102; $P = 0.004$) treatments compared with the placebo (5383 kJ; 95% CI: 4722, 6045). A

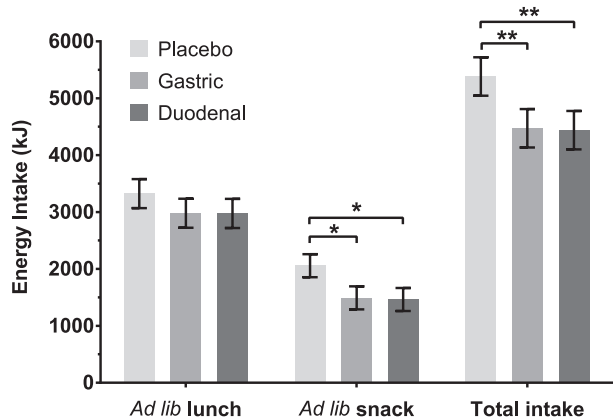


FIGURE 2 The effect of treatment on ad libitum energy intake (kJ) at the outcome lunch (12:00 h), snack (14:00 h), and the combined intake (Total intake). Treatments comprised either a vehicle control (Placebo) or a formulated hops extract designed to release in the stomach (Gastric) or in the proximal small intestine (Duodenum). Analysis was conducted using the Mixed procedure (SAS 9.4) with treatment, visit number, and treatment order as factors. A significant effect of treatment was observed for both the snack ($P = 0.027$) and for total intake ($P = 0.006$). Fisher's least significant difference (LSD) post hoc pairwise analysis demonstrated a significant reduction in energy intake for both the gastric ($P = 0.022$) and duodenal ($P = 0.017$) treatments compared with the placebo treatment at the snack and when assessed as total intake ($P = 0.006$ and $P = 0.004$, respectively). Values are means ± SEMs ($n = 19$). * $P < 0.05$. ** $P < 0.01$.

significant effect of treatment ($F_{2,34} = 4.0$, $P = 0.027$) was also observed at the ad libitum snack with a reduction of EI in both the gastric (1492 kJ; 95% CI: 1095, 1889; $P = 0.022$) and duodenal (1463 kJ; 95% CI: 1066, 1861; $P = 0.017$) treatments compared with placebo (2058 kJ; 95% CI: 1661, 2455). There was no evidence of a significant effect of treatment on EI at the ad libitum lunch for either gastric (2980 kJ; 95% CI: 2481, 3480; $P = 0.192$) or duodenal (2976 kJ; 95% CI: 2476, 3477; $P = 0.188$) treatments compared with the placebo (3325 kJ; 95% CI: 2825, 3825).

Blood parameters

Ghrelin, CCK, GLP-1, and PYY.

The effects of treatment on plasma concentrations and $AUC_{0-270 \text{ min}}$ responses of the appetite-regulating hormones ghrelin, CCK, GLP-1, and PYY are shown in **Figure 3A–D**. CCK and GLP-1 values were log-transformed for analysis and presented back-transformed. All 4 peptide hormone profiles exhibited a highly significant effect of time ($P < 0.001$) with predictable changes driven primarily by the timing of meals.

Ghrelin. Plasma concentrations of the orexigenic hormone ghrelin exhibited a significant treatment × time interaction ($F_{30,544} = 1.73$, $P = 0.010$). Subsequent post hoc analysis demonstrated a significant increase in ghrelin immediately prior to the ad libitum lunch at $T = 45 \text{ min}$ (47.7 pg/mL; 95% CI: 36.3, 59.1; $P = 0.013$) and $T = 60 \text{ min}$ (56.1 pg/mL; 95% CI: 44.7, 67.5; $P = 0.001$) for the duodenal treatment and at $T = 60 \text{ min}$ for the gastric treatment (58.7 pg/mL; 95% CI: 47.3, 70.0; $P < 0.001$) compared with the placebo ($T = 45 \text{ min}$: 33.1 pg/mL; 95% CI: 21.7, 44.4 and $T = 60 \text{ min}$: 36.7 pg/mL, 95% CI: 25.4, 48.1). No significant differences were detected between any of the treatments at any time point after lunch. There was also no evidence for a significant effect of treatment on ghrelin $AUC_{0-270 \text{ min}}$ responses to gastric (9883 pg/mL·min; 95% CI: 7308, 12,458; $P = 0.573$) or duodenal (10,349 pg/mL·min; 95% CI: 7774, 112,924; $P = 0.305$) treatments compared with placebo (9322 pg/mL·min; 95% CI: 6749, 11,895) (**Figure 3A**).

CCK. A significant main effect of treatment ($F_{2,52} = 4.8$, $P < 0.012$) was observed for plasma concentrations of the anorexigenic hormone CCK (**Figure 3B**). Post hoc analysis demonstrated that plasma CCK concentrations were significantly increased in the gastric treatment at $T = 90$ (4.8 pM; 95% CI: 3.7, 6.2; $P < 0.001$) and $T = 150 \text{ min}$ (3.2 pM; 95% CI: 2.5, 4.2; $P = 0.019$) compared with placebo (2.9 pM; 95% CI: 2.2, 3.7 and 2.4 pM; 95% CI: 1.8, 3.0, respectively). Duodenal CCK concentrations were generally similar to the gastric treatment, except for $T = 90 \text{ min}$, when the duodenal treatment (3.6 pM; 95% CI: 2.8, 4.7; $P = 0.036$) was significantly lower than the gastric treatment, and no significant differences were detected between the duodenal and placebo treatments at any time point.

A significant effect of treatment was also seen for the CCK $AUC_{0-270 \text{ min}}$ responses ($F_{2,32} = 13.5$, $P < 0.001$), with increased hormone secretion observed in both the duodenal (777 pM·min; 95% CI: 639, 944; $P < 0.001$) and gastric (812 pM·min; 95% CI: 667, 987; $P < 0.001$) treatments compared with the placebo

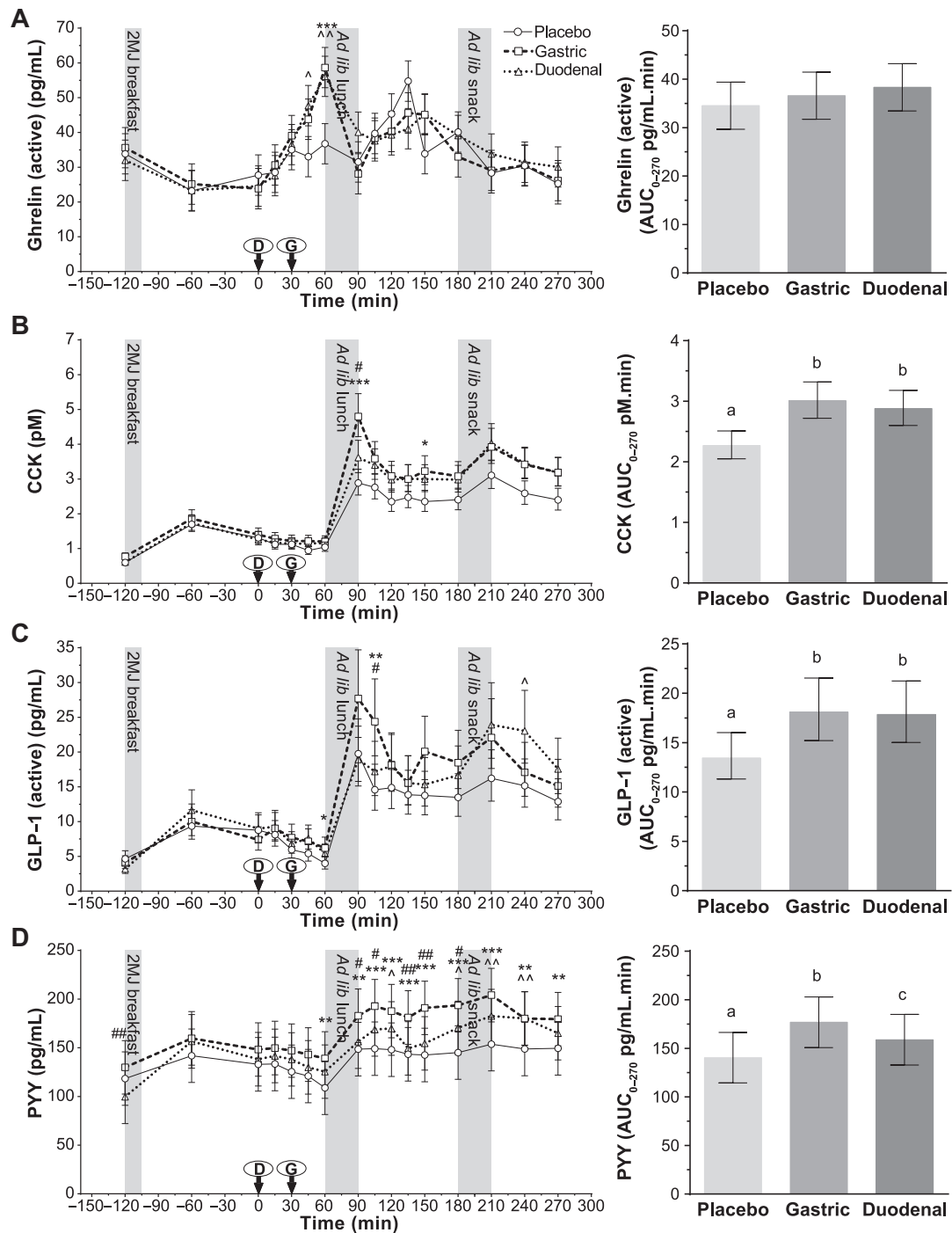


FIGURE 3 Plasma concentrations of (A) ghrelin (active), (B) cholecystikinin (CCK), (C) glucagon-like peptide 1 (active) (GLP-1), and (D) peptide YY (PYY) following administration of a control (Placebo) or a formulation containing hop extract targeted to either the small intestine (Duodenal) or stomach (Gastric) using delayed-release or standard capsules, respectively. Arrows indicate capsule administration; gray bars indicate the time allowed for the 2-MJ fixed-energy breakfast and the ad libitum lunch and snack. Analysis was conducted using the mixed procedure (SAS 9.4) with treatment, time, visit number, and treatment order as factors. Significant main effects of treatment were observed for A ($P = 0.010$), B ($P < 0.012$), C ($P = 0.023$), and D ($P < 0.001$) hormone time profiles. Significant Fisher's least significant difference post hoc pairwise comparisons are shown: gastric compared with placebo ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$), duodenal compared with placebo ($^{\wedge}P < 0.05$, $^{\wedge\wedge}P < 0.01$, $^{\wedge\wedge\wedge}P < 0.001$), and gastric compared with duodenal ($^{\#}P < 0.05$, $^{\#\#\#}P < 0.01$, $^{\#\#\#\#}P < 0.001$). Histograms show effect of treatment on $AUC_{0-270 \text{ min}}$ for each hormone. Analysis was conducted using the mixed procedure (SAS 9.4) with treatment, visit number, and treatment order as factors. A significant effect of treatment was observed for B ($P < 0.001$), C ($P = 0.004$), and D ($P < 0.001$) only, with letters denoting significantly ($P < 0.05$) different means. Values are means \pm SEMs; $n = 18$. *Ad lib*, ad libitum.

treatment (612 pM·min; 95% CI: 504, 745) (Figure 3B). Gastric and duodenal treatments did not differ significantly from each other in AUC_{0-270 min} responses.

GLP-1. Plasma concentrations of the insulin secretagogue and anorexigenic hormone GLP-1 exhibited a significant treatment ($F_{2,93} = 3.9$, $P = 0.023$) and treatment \times time interaction ($F_{30,481} = 1.5$, $P = 0.044$) despite considerable interindividual variability [including 1 individual who exhibited baseline (T = -120 min) concentrations $\sim 8\times$ the average]. Post hoc analysis demonstrated that immediately prior to the lunch (T = 60 min), GLP-1 concentrations were significantly higher in the gastric (6.2 pg/mL; 95% CI: 4.0, 9.6; $P = 0.012$) compared with placebo (4.0 pg/mL; 95% CI: 2.6, 6.2) treatment. The gastric treatment also exhibited an enhanced postprandial response to the ad libitum lunch, reaching statistical significance at T = 105 min (24.4 pg/mL; 95% CI: 15.7, 37.9; $P = 0.003$) compared with the placebo (14.6 pg/mL; 95% CI: 9.4, 22.7) and duodenal (17.2 pg/mL; 95% CI: 11.1, 26.7; $P = 0.047$) treatments (Figure 3C). Interestingly, the duodenal treatment elicited a significantly enhanced postprandial response compared with the placebo treatment only following the later snack at T = 240 min (23.0 pg/mL; 95% CI: 14.8, 35.8 compared with 15.1 pg/mL; 95% CI: 9.7, 23.6; $P = 0.018$, respectively). A significant effect of treatment was also seen for GLP-1 AUC_{0-270 min} responses ($F_{2,32} = 6.5$, $P = 0.004$), with increased hormone secretion observed in both the duodenal (4822 pg/mL·min; 95% CI: 3429, 6780; $P = 0.005$) and gastric (4884 pg/mL·min; 95% CI: 3474, 6868; $P = 0.003$) treatments compared with the placebo treatment (3633 pg/mL·min; 95% CI: 2584, 5107) (Figure 3C).

PYY. A significant effect of treatment ($F_{2,68.3} = 12.7$, $P < 0.001$) was observed for plasma concentrations of the anorexigenic gut hormone PYY (Figure 3D), although considerable interindividual variability was observed (2 participants had baseline values 3–4 \times the average). Post hoc analysis demonstrated that compared with the placebo, gastric delivery of hop extract produced significant increases in PYY immediately prior to the lunch (T = 60, $P = 0.007$), with differences becoming more apparent after lunch through to the end of the session (T = 90–270 min, $P < 0.01$). The PYY response to the duodenal treatment was generally less than that observed for the gastric treatment, with significantly ($P < 0.05$) reduced responses observed at T = -120, 90, 105, 135, 150, and 180 min. However, relative to placebo treatment, concentrations were significantly ($P < 0.05$) increased at T = 120, 180, 210, and 240 min.

A significant effect of treatment was seen for the PYY AUC_{0-270 min} responses ($F_{2,32} = 11.1$, $P < 0.001$). Compared with placebo PYY AUC_{0-270 min} responses (37,907 pg/mL·min; 95% CI: 24,121, 51,693), both gastric (47,758 pg/mL·min; 95% CI: 33,970, 61,546; $P < 0.001$) and, to a lesser extent, duodenal (42,901 pg/mL·min; 95% CI: 29,113, 56,689, $P = 0.023$) treatments were significantly increased. PYY release was also significantly greater ($P = 0.027$) in the gastric compared with duodenal treatments (Figure 3D).

Glucose, insulin, GIP, and PP.

Effects of treatment on plasma concentrations and AUC_{0-270 min} responses of glucose, insulin, GIP, and PP are

shown in Figure 4A–D. All 4 profiles exhibited a highly significant effect of time ($P < 0.001$) with predictable changes driven primarily by the timing of meals.

Glucose. Changes in glucose with time (Figure 4A) suggest that both gastric and duodenal treatments delayed the postprandial hyperglycemia peak following the ad libitum lunch (T = 90 min: 5.1 mM; 95% CI: 4.7, 5.5 and 5.1 mM; 95% CI: 4.7, 5.5, respectively) compared with placebo (T = 90 min: 5.7 mM; 95% CI: 5.3, 6.2). However, Fisher's protected LSD post hoc analysis could not be conducted as there was no significant main effect of treatment or treatment \times time interaction. In addition, no significant differences were detected in the glucose AUC_{0-270 min} response between placebo (1356 mM·min; 95% CI: 1285, 1426), gastric (1345 mM·min; 95% CI: 1274, 1416), and duodenal (1368 mM·min; 95% CI: 1297, 1438) treatments (Figure 4A).

Insulin. Plasma insulin concentrations exhibited a significant effect of treatment ($F_{2,89} = 11.2$, $P < 0.001$) and a treatment \times time ($F_{30,465} = 1.6$, $P = 0.033$) interaction (Figure 4B) following log transformation. Post hoc analysis demonstrated that insulin responses to the ad libitum lunch and snack showed a similar significant reduction ($P < 0.05$) following the gastric and duodenal treatments from T = 90 to 240 min compared with the placebo. Insulin responses in the gastric and duodenal treatments did not differ significantly from each other at any time point. A highly significant effect of treatment ($F_{2,32} = 10.8$, $P < 0.001$) was also observed in nontransformed insulin AUC_{0-270 min} responses (Figure 4B), with a reduction in postprandial insulin secretion following the gastric (343,308 pg/mL·min; 95% CI: 275,223, 411,393; $P = 0.001$) and duodenal (320,865 pg/mL·min; 95% CI: 252,780, 388,950; $P < 0.001$) treatments compared with the placebo treatment (437,190 pg/mL·min; 95% CI: 369,168, 505,212). Insulin AUC_{0-270 min} responses in the gastric and duodenal treatments did not differ significantly from each other.

GIP. Plasma concentrations of the insulin secretagogue GIP exhibited a significant effect of treatment ($F_{2,67} = 6.8$, $P = 0.002$) and a treatment \times time ($F_{30,544} = 1.7$, $P = 0.010$) interaction. Post hoc analysis demonstrated that the postprandial response to the ad libitum lunch and snack was significantly reduced in both the gastric and duodenal treatments compared with the placebo treatment from T = 90 to 240 min ($P < 0.050$). Gastric and duodenal treatments did not differ significantly from each other at any time point. GIP AUC_{0-270 min} responses (Figure 4C) exhibited a highly significant effect of treatment ($F_{2,32} = 15.5$, $P < 0.001$), with reductions in postprandial GIP secretion following both the gastric (86,687 pg/mL·min; 95% CI: 72,072, 101,302; $P < 0.001$) and duodenal (90,369 pg/mL·min; 95% CI: 75,754, 104,984; $P < 0.001$) treatments compared with the placebo treatment (115,732 pg/mL·min; 95% CI: 101,130, 130,334). Gastric and duodenal treatments did not differ significantly from each other.

PP. Plasma concentrations of the pancreatic hormone PP exhibited a significant effect of treatment ($F_{2,72} = 8.7$, $P < 0.001$) and a treatment \times time interaction ($F_{30,525} = 1.91$, $P = 0.003$), increasing following meals in all treatment groups (Figure 4D). Post hoc analysis demonstrated that postprandial PP responses

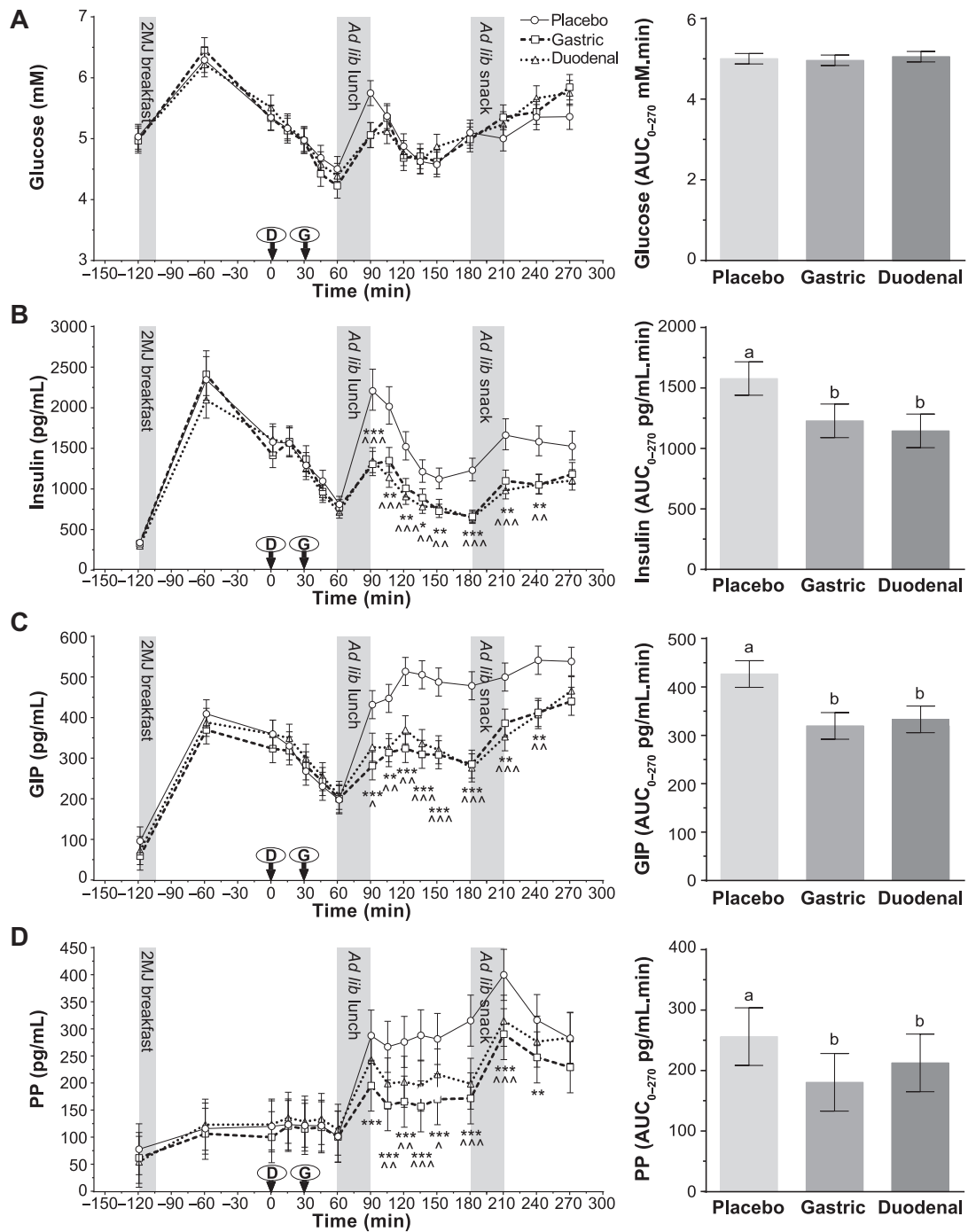


FIGURE 4 Plasma concentrations of (A) glucose, (B) insulin, (C) glucose-dependent insulinotropic polypeptide (GIP), and (D) pancreatic polypeptide (PP) following administration of a control (Placebo) or a formulation containing hop extract targeted to either the small intestine (Duodenal) or stomach (Gastric) using delayed-release or standard capsules, respectively. Arrows indicate capsule administration; gray bars indicate the time allowed for the 2-MJ fixed-energy breakfast and the ad libitum lunch and snack. Analysis was conducted using the mixed procedure (SAS 9.4) with treatment, time, visit number, and treatment order as factors. A significant main effect of treatment was observed for B ($P < 0.001$), C ($P = 0.002$), and D ($P < 0.001$) only. Significant Fisher's least significant difference post hoc pairwise comparisons are shown: gastric compared with placebo ($^{\wedge}P < 0.05$, $^{\wedge\wedge}P < 0.01$, $^{\wedge\wedge\wedge}P < 0.001$), and gastric compared with duodenal ($^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$). Histograms show effect of treatment on $AUC_{0-270 \text{ min}}$ for each hormone. Analysis was conducted using the mixed procedure (SAS 9.4) with treatment, visit number, and treatment order as factors. A significant effect of treatment was observed for B ($P < 0.001$), C ($P < 0.001$), and D ($P < 0.001$) only, with letters denoting significantly ($P < 0.05$) different means. Values are means \pm SEMs; $n = 18$. *Ad lib*, ad libitum.

were significantly ($P < 0.05$) reduced in both the gastric ($T = 90$ – 240 min) and duodenal ($T = 105$ – 210 min) treatments compared with the placebo treatment. Duodenal and gastric treatments did not differ significantly from each other at any time point.

A highly significant effect of treatment ($F_{2,32} = 11.6$, $P < 0.001$) was seen for the PP $AUC_{0-270\text{ min}}$ responses, with reduced hormone secretion observed in both the duodenal (56,464 pg/mL·min; 95% CI: 31,729, 81,199; $P < 0.001$) and gastric (47,966 pg/mL·min; 95% CI: 23,231, 72,701; $P < 0.001$) treatments compared with the placebo treatment (67,977 pg/mL·min; 95% CI: 43,246, 92,708) (Figure 4D). Gastric and duodenal treatments did not differ significantly from each other.

VAS—appetite.

Effects of treatment on the subjective ratings of hunger, fullness, prospective consumption, satiety, and thirst over time and as $AUC_{0-300\text{ min}}$ are shown in Figure 5A–E. All 5 profiles exhibited a highly significant effect of time ($P < 0.001$) with predictable patterns driven by meal timing. However, there was no evidence for a significant main effect of treatment or treatment \times time interaction for any of the changes in VAS appetite profiles or in $AUC_{0-300\text{ min}}$.

VAS—vitality.

Effects of treatment on subjective ratings of energy and relaxation are shown in Supplemental Figure 3A–B. Ratings of energy exhibited a significant treatment effect ($F_{2,105} = 3.5$, $P = 0.033$), with post hoc analysis demonstrating significantly lower energy ratings in the duodenal treatment at $T = 120$ (40 mm; 95% CI: 31, 50) compared with both the gastric (48 mm; 95% CI: 38, 57; $P = 0.021$) and placebo (53 mm; 95% CI: 44, 63; $P < 0.001$) treatments. Significantly lower energy ratings were also observed at $T = 150$ min for both the duodenal (41 mm; 95% CI: 32, 51; $P = 0.005$) and gastric (43 mm; 95% CI: 34, 53; $P = 0.025$) treatments compared with placebo treatment (51 mm; 95% CI: 41, 60). A significant treatment effect ($F_{2,34} = 4.98$, $P = 0.013$) was also seen for $AUC_{0-300\text{ min}}$ responses, with lower ratings for both the gastric (13,907 mm·min; 95% CI: 11,175, 16,639; $P = 0.045$) and duodenal (13,410 mm·min; 95% CI: 10,678, 16,142; $P = 0.004$) treatments compared with the placebo treatment (14,923 mm·min; 95% CI: 12,191, 17,655) (Supplemental Figure 3A).

Subjective ratings of relaxation exhibited a significant treatment effect ($F_{2,105} = 3.5$, $P = 0.033$). However, post hoc analysis identified only the final time point ($T = 300$) as significantly different between gastric (64 mm; 95% CI: 55, 73; $P = 0.027$) or duodenal (63 mm; 95% CI: 54, 73; $P = 0.044$) compared with placebo (72 mm; 95% CI: 63, 81) treatment (Supplemental Figure 3B). No significant effect of treatment was observed for $AUC_{0-300\text{ min}}$ responses.

VAS—GI discomfort.

Effects of treatment on subjective ratings of nausea, urge to vomit, bloating, abdominal discomfort, and heartburn are shown in Figure 6A–E. All GI discomfort ratings were log-transformed

for analysis and presented back-transformed. Only bloating and heartburn time profiles exhibited a significant effect of time ($P \leq 0.034$).

Nausea. Ratings of nausea (Figure 6A) exhibited a significant treatment effect ($F_{2,148} = 11.6$, $P < 0.001$), with post hoc analysis demonstrating significantly ($P < 0.050$) higher nausea ratings in the duodenal treatment at $T = 90$ and 120–270 min and in the gastric treatment at $T = 90$, 135, and 180 min, compared with the placebo. Significant differences between the gastric and duodenal treatments were seen at $T = 210$ – 270 min. A significant treatment effect ($F_{2,34} = 11.7$, $P < 0.001$) on the nausea $AUC_{0-300\text{ min}}$ response was also observed, with higher ratings in the gastric (533 mm·min; 95% CI: 317, 964; $P = 0.008$) and duodenal (948 mm·min; 95% CI: 543, 1654; $P < 0.001$) treatments than in the placebo treatment (259 mm·min; 95% CI: 149, 452).

Urge to vomit. A significant main effect of treatment ($F_{2,154} = 6.73$, $P = 0.002$) was seen in the urge to vomit profile (Figure 6B), with significant ($P < 0.050$) increases at $T = 105$, 120, and 180 min for the duodenal treatment compared with placebo treatment. The duodenal and gastric treatments also differed significantly ($P = 0.009$) at $T = 120$ min. A significant treatment effect ($F_{2,34} = 4.02$, $P = 0.027$) on $AUC_{0-300\text{ min}}$ responses was also seen, with an increase in the urge to vomit in the duodenal (533 mm·min; 95% CI: 317, 964; $P = 0.008$) compared with the placebo (177 mm·min; 95% CI: 92, 338) treatment. The gastric treatment (258 mm·min; 95% CI: 360, 185) did not differ significantly from either placebo or duodenal treatment.

Bloating. A significant main effect of treatment ($F_{2,143} = 15.3$, $P < 0.001$) was seen in the time profile of subjective ratings of abdominal bloating (Figure 6C). Post hoc analysis demonstrated significantly ($P < 0.050$) higher ratings of abdominal bloating in the duodenal treatment at $T = 15$ and 45–240 min, as well as in the gastric treatment at $T = 90$, 105, and 135 min, than in the placebo treatment (Figure 6C). The duodenal and gastric treatments also differed significantly ($P < 0.050$) at $T = 120$, 180, and 240 min. A significant treatment effect ($F_{2,34} = 8.99$, $P < 0.001$) was also seen in $AUC_{0-300\text{ min}}$ responses, with an increase in bloating in the duodenal (1157 mm·min; 95% CI: 600, 2231; $P < 0.001$) and gastric (607 mm·min; 95% CI: 315, 1168; $P = 0.016$) treatments compared with the placebo treatment (231 mm·min; 95% CI: 120, 445).

Abdominal discomfort. A significant main effect of treatment ($F_{2,135} = 8.79$, $P < 0.001$) was seen for ratings of abdominal discomfort (Figure 6D), with post hoc analysis demonstrating significantly ($P < 0.010$) higher abdominal discomfort ratings in the duodenal treatment at $T = 90$, 105, 150, and 240 min, as well as in the gastric treatment ($P < 0.050$) at $T = 105$ and 150 min, compared with placebo. However, there was no evidence for a significant effect of treatment on $AUC_{0-300\text{ min}}$.

Heartburn. No significant main effects of treatment or treatment \times time interactions were observed for the profile of ratings of heartburn or $AUC_{0-300\text{ min}}$ responses (Figure 6E). There was also no evidence of a significant correlation between any $AUC_{0-300\text{ min}}$ measures of GI discomfort (nausea, urge to vomit,

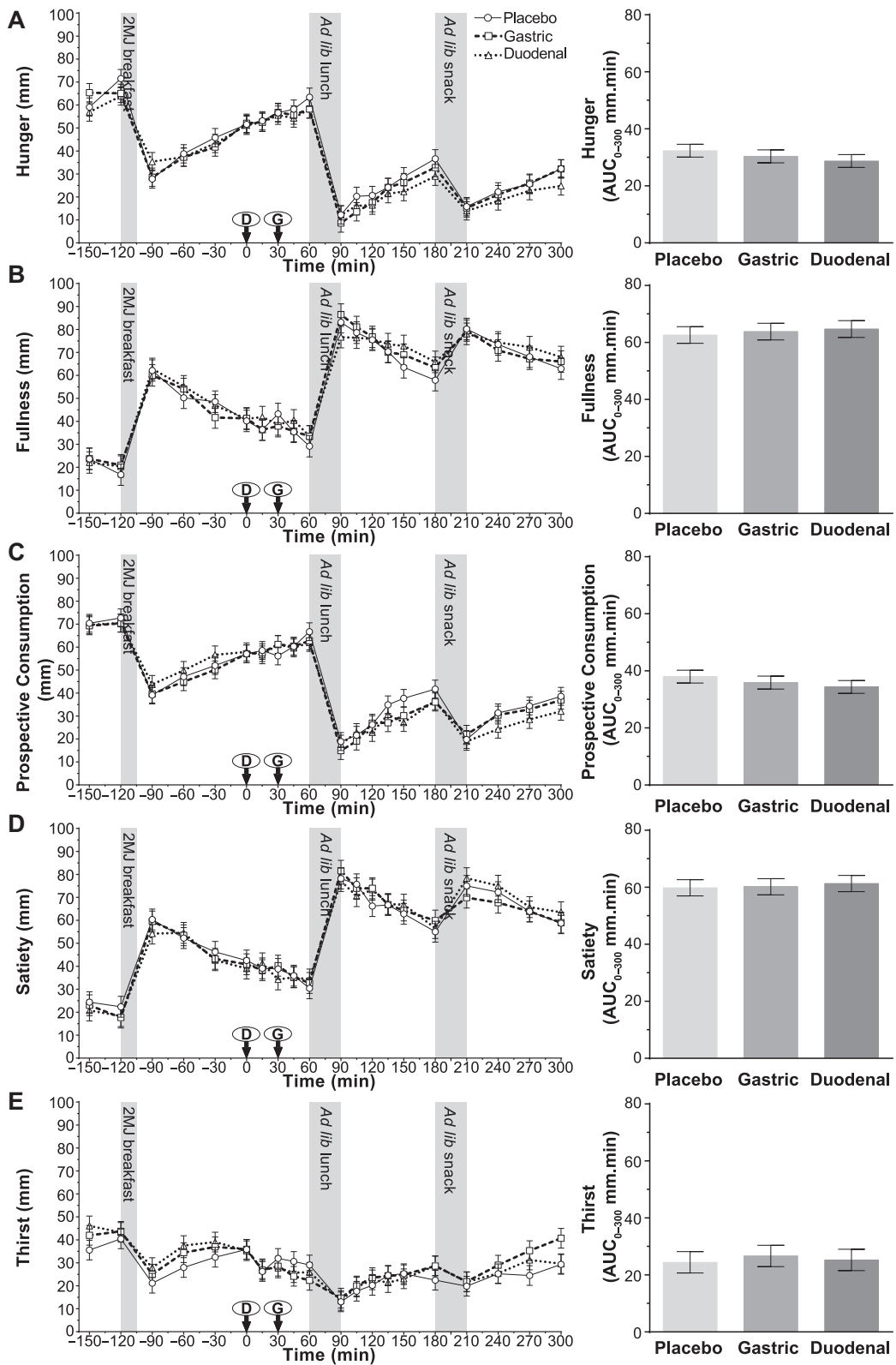


FIGURE 5 Visual analog scale (VAS) ratings of (A) hunger, (B) fullness, (C) prospective consumption, (D) satiety, and (E) thirst following administration of a control (Placebo) or a formulation containing hop extract targeted to either the small intestine (Duodenal) or stomach (Gastric) using delayed-release or standard capsules, respectively. Arrows indicate capsule administration; gray bars indicate the time allowed for the 2-MJ fixed-energy breakfast and the ad libitum lunch and snack. Analysis was conducted using the mixed procedure (SAS 9.4) with treatment, time, visit number, and treatment order as factors. No main effect of treatment or a treatment \times time interaction was observed for any measure. Histograms show mean AUC_{0-300 min} for each VAS measure from 0 to 300 min. Analysis was conducted using the mixed procedure (SAS 9.4) with treatment, visit number, and treatment order as factors. No significant effects of treatment were seen. Values are means \pm SEMs; $n = 19$. *Ad lib*, ad libitum.

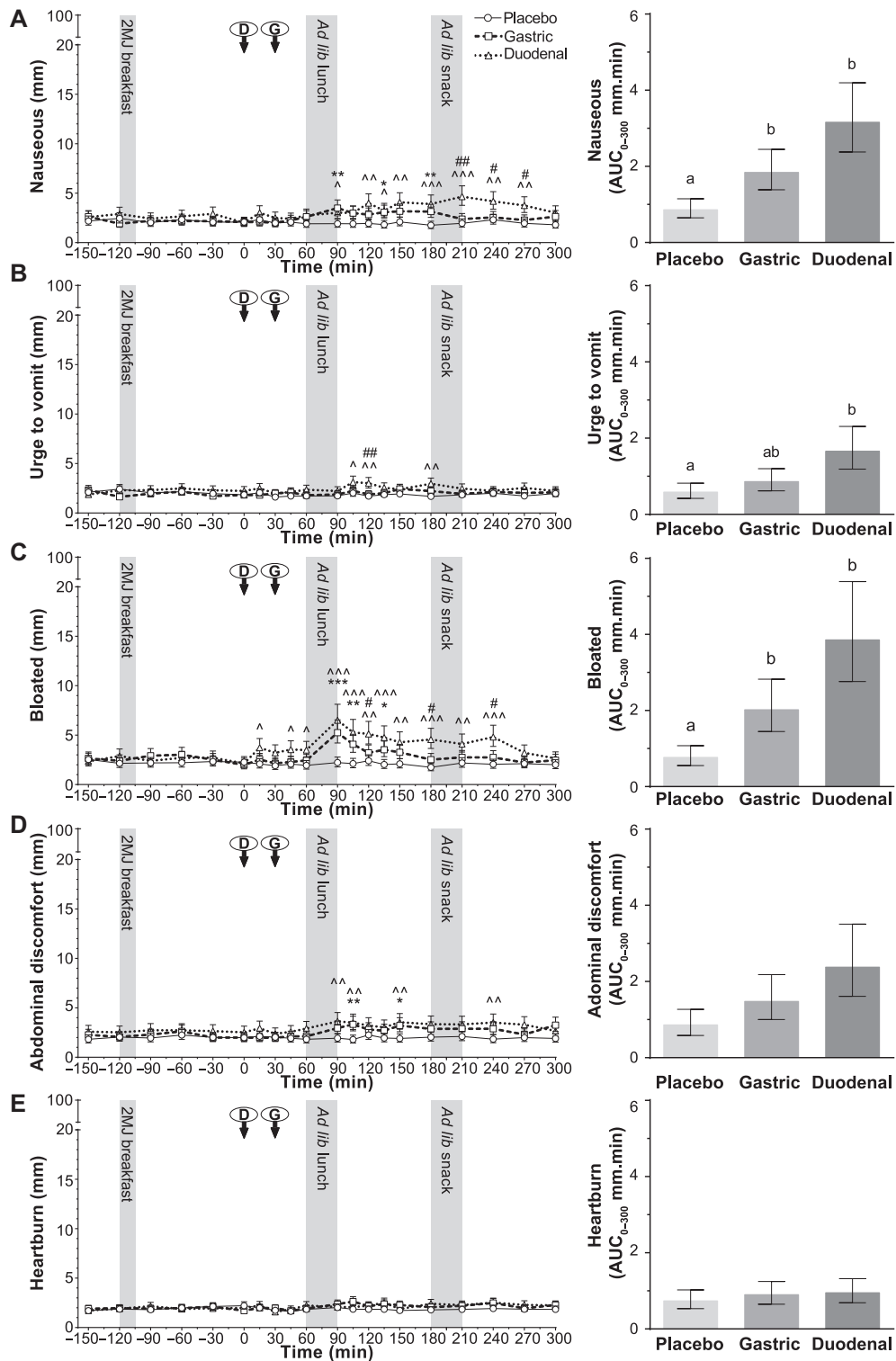


FIGURE 6 Visual analog scale (VAS) ratings of (A) nausea, (B) urge to vomit, (C) bloating, (D) abdominal discomfort, and (E) heartburn following administration of a control (Placebo) or a formulation containing hop extract targeted to either the small intestine (Duodenal) or stomach (Gastric) using delayed-release or standard capsules, respectively. Arrows indicate capsule administration; gray bars indicate the time allowed for the 2-MJ fixed energy breakfast and the ad libitum lunch and snack. Analysis was conducted using the mixed procedure (SAS 9.4) with treatment, time, visit number, and treatment order as factors. A significant main effect of treatment was observed for A ($P < 0.001$), B ($P = 0.002$), C ($P < 0.001$), and D ($P < 0.001$) only. Significant Fisher's least significant difference post hoc pairwise comparisons are shown: gastric compared with placebo (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$), duodenal compared with placebo ($\hat{P} < 0.05$, $\hat{\hat{P}} < 0.01$, $\hat{\hat{\hat{P}}} < 0.001$), and gastric compared with duodenal ($\#P < 0.05$, $\#\#\#P < 0.01$). Histograms show effect of treatment on $AUC_{0-300 \text{ min}}$ for each VAS scale. Analysis was conducted using the mixed procedure (SAS 9.4) with treatment, visit number, and treatment order as factors. A significant main effect of treatment on $AUC_{0-300 \text{ min}}$ was observed for A ($P < 0.001$), B ($P = 0.027$), and C ($P < 0.001$) only, with letters denoting significantly ($P < 0.05$) different means. Values are means \pm SEMs; $n = 19$. *Ad lib*, ad libitum.

TABLE 2 The effects of treatment on numbers of reported adverse event symptoms and range of self-reported intensities¹

| Characteristic | Placebo | Gastric | Duodenal |
|-------------------------|---------|----------------------|----------------------------|
| Nausea | | 2/19 moderate-severe | |
| Loose stool/diarrhea | | 6/19 mild-moderate | |
| Stomach rumbling | | 1/19 mild | |
| Upset stomach | | 1/19 mild | |
| Bloating | | 2/19 moderate-severe | |
| Headache | | 1/19 mild | |
| Frequency of defecation | | | 1/19 moderate ² |

¹Severity of adverse events was reported using a 3-point scale of mild, moderate, or severe over the study visit and washout period for each treatment. Treatments comprised a vehicle control (Placebo) or hop extract formulation targeted to either the small intestine (Duodenal) or stomach (Gastric) using delayed-release or standard capsules, respectively.

²One participant noted a reduced frequency of defecation over the following week washout period.

bloating, abdominal discomfort, or heartburn) and total EI for any treatment (**Supplemental Table 5**).

VAS—meal palatability.

There was no evidence for a main effect of treatment on VAS ratings of pleasantness, visual appeal, smell, taste, aftertaste, or overall palatability for the fixed-energy breakfast or the ad libitum lunch and snack outcome meals (**Supplemental Figure 4**).

POMS.

The effect of treatment on the 6 POMS mood subscales and the computed total mood disturbance score measured prior to (pre) and following treatment administration (post) are shown in **Supplemental Figure 5A–G**. Tension–anxiety, depression–dejection, and anger–hostility subscales required log-transformation for analysis and are presented back-transformed.

Only vigor–activity and fatigue–inertia subscales exhibited a significant overall effect of time ($P < 0.001$) with post scores significantly lower (-3.3 ; 95% CI: $-4.5, -2.1$) and higher (2.2 ; 95% CI: $1.1, 3.3$) than pre scores, respectively. Only depression–dejection ($F_{2,88} = 3.3, P = 0.041$) and anger–hostility ($F_{2,88} = 4.83, P = 0.010$) exhibited a significant main effect of treatment. Post hoc analysis demonstrated a significant increase in scoring of depression–dejection following administration of the gastric (2.1 ; 95% CI: $1.4, 3.1$; $P = 0.022$) compared with placebo (1.4 ; 95% CI: $1.0, 2.1$) treatment. (**Supplemental Figure 5B**). Significant increases in ratings of anger–hostility were also seen following the gastric treatment compared with duodenal ($0.7 \pm 1.2, P = 0.019$) and placebo ($1.5 \pm 1.2, P = 0.007$) ratings posttreatment (**Supplemental Figure 5C**).

Adverse events.

The numbers of participants reporting adverse event symptoms, such as loose stool/diarrhea, nausea, rumbling or upset stomach, bloating, and headache during the study day, and their subjective ratings of severity (mild, moderate, or severe) are shown in **Table 2**. The primary analysis of all 19 participants revealed a total of 14 mild to severe adverse event symptoms

reported by 8 participants, the majority of which (93%) occurred while on the gastric treatment. No adverse events were reported while on the placebo treatment, and only 1 individual reported a reduced frequency of defecation in the week following the duodenal treatment (washout period), which may not have been attributable to the treatment, given the delay.

Discussion

GI delivery of a bitter hop extract significantly decreased energy intake and increased appetite-suppressing CCK, PYY, and GLP-1 plasma concentrations. These changes occurred without significant effects on subjective measures of appetite or the hedonic properties of the test meals. However, they were accompanied by increases in subjective ratings of GI discomfort (e.g., nausea, bloating, urge to vomit, and abdominal discomfort), known side effects of administration of gut peptide hormones or their antagonists (72, 73). Although these GI discomfort responses are expected to decrease EI and may be confounders, we found no correlation between EI and any measure of GI discomfort. This is in agreement with a previous study investigating the relation between CCK-8 infusion, nausea, and EI (73) in which the authors concluded that “although feelings of anxiety and nausea may accompany CCK infusions, they are not necessary for the effects of CCK on appetite.” The magnitude of total EI suppression (17%) is significant in the context of weight management applications (74) and compares favorably with results from previous studies in humans (0–22%) that have used encapsulation, intragastric, or intraduodenal delivery of a variety of bitter tastants (38, 40, 42, 43, 47, 75, 76).

The current study supports a mechanism of action involving enhanced and sustained release of the anorexigenic gut hormones CCK, GLP-1, and PYY from intestinal EECs. All 3 gut peptide hormones play a key role in the homeostatic regulation of energy intake, appetite, and GI function [reviewed in Steinert et al. (77)], including delay of gastric emptying (78–80), and have been shown previously to respond to T2R ligands (29, 30, 45, 49). Maximum postprandial increases in CCK following hop treatments were 6-fold that of baseline and in the upper range reported for dietary interventions (0.5- to 7.9-fold) (81). A similar postprandial increase was observed following hop treatments

for GLP-1 (6.4-fold) with a smaller fold change for PYY (1.7-fold). A recent meta-analysis of CCK, GLP-1, and PYY infusion studies (81) proposed that the minimum fold changes required to decrease ad libitum energy intake were 3.6-, 4.0-, and 3.1-fold, respectively.

A significant enhancement of the orexigenic hormone ghrelin response prior to the lunch was also seen for both gastric and duodenal targeting of the hop extract. This is consistent with the duodenum also being a source of ghrelin secretion, second only to the stomach (82, 83), although pyloric reflux may also play a role (84). Gavage of T2R agonists has also been shown to stimulate the secretion of ghrelin in mice, resulting in a temporary increase in food intake (85). However, our results contrast with several recent reports of either unchanged or suppressed ghrelin following intragastric infusion of T2R agonists (quinine, denatonium benzoate) in humans (41, 44, 86), indicating potential T2R specificity in this response. The mechanism(s) by which T2R agonists stimulate ghrelin secretion are poorly understood, as gastric ghrelin-secreting cells are of the closed type and do not directly contact the GI lumen.

It is also noteworthy that there was no significant treatment-induced difference in VAS measurements relating to appetite despite the significant decrease in energy intake seen with both hop treatments. Although correlations between subjective assessments (e.g., hunger) and behavioral effects (e.g., energy intake) are often observed, they assess fundamentally different things, have been reported to show weak correlations, and do not always concur (68, 87). Previous studies using either gastric or duodenal delivery of T2R agonists have shown effects on subjective measures of appetite in both men (88–90), and women (41, 91), although many studies show no response (43, 45–47). Interestingly, participants in the current study did achieve similar feelings of fullness at the ad libitum test meals after consuming less food when taking both hop treatments compared with the placebo. Viewed in this context, treatment with hop extract may modulate early satiety, which is associated with impaired gastric accommodation and gastric emptying (92).

Glucoregulatory hormones (e.g., GLP-1, GIP, insulin) and the slowing of gastric emptying are key determinants of the postprandial glycemic response. Bitter tastants have been shown to stimulate the secretion of the incretin hormone GLP-1 from EEC cell lines (30, 49), whereas in mice, gavage of bitter gourd extract (93) or denatonium benzoate (30) stimulates GLP-1 and subsequent insulin secretion, leading to lowering of blood glucose. A recent study in healthy men also demonstrated that intragastric and intraduodenal administration of the bitter tastant quinine similarly lowered plasma glucose, increased plasma insulin and GLP-1, and slowed gastric emptying (40). The current data also demonstrate an enhancement in the postprandial GLP-1 response to the lunch following gastric treatment and to the later snack following duodenal targeting of hop extract. However, this response was accompanied in both hop treatments by a similar reduction in the postprandial insulin response for both test meals. Interestingly, GIP, the only gut peptide hormone measured that is secreted from the enteroendocrine K-cell subtype, also exhibited a similar reduction in postprandial response following both hop treatments. This is in marked contrast to the observed stimulation of CCK, GLP-1, and PYY producing EECs by hop extract, suggesting that K cells lack the appropriate T2Rs. GIP has been shown to be responsible for the majority of the incretin

effect in healthy individuals, affecting glucose concentrations during the whole postprandial period (94). In contrast, GLP-1 primarily affects glycemic regulation in the early postprandial phase, delaying gastric emptying and reducing plasma glucagon concentrations (94). GIP has also recently been demonstrated as a PP secretagogue (94, 95). Hence, the suppression of postprandial GIP in the hop treatment groups may in part explain the suppression of insulin and PP observed. In addition, delays in gastric emptying and subsequent effects on nutrient absorption may also account for the observed reduction in these hormones (96). Despite this, postprandial glycemia was not adversely affected compared with placebo, indicating a possible metabolic shift toward greater insulin sensitivity, a possible consequence of increased GLP-1 secretion. Replication of these results using a fixed-energy meal would remove any influence from the intertreatment differences in absolute energy intake that occurred at the ad libitum meals.

Off-target effects of hop extract included significant increases in subjective ratings of GI discomfort consistent with known effects of CCK, GLP-1, and PYY on upper GI sensations (72, 97). In addition, gastric treatment induced significant changes in several negative mood state subscales (depression–dejection, anger–hostility), which may reflect the prevalence of adverse events with this particular treatment. The known sedative activity of hop bitter acids may also have contributed to the decline in subjective ratings of energy following hop treatment (98, 99). Interestingly, most reported adverse events were associated with the gastric treatment. Targeting delivery to the small intestine improved tolerance of the hop treatment, suggesting that gastric T2Rs may play a key role in detection of ingested toxins, stimulating a host defense mechanism involving net secretion of fluid and electrolytes into the intestinal lumen, accelerating intestinal transit to flush harmful compounds from the GI tract in a process similar to that described for T2Rs in the human and rat large intestine (100). Further optimization of the dosage of hops extract used and its timing relative to meals may also contribute to a reduction in the side effect profile.

The supercritical CO₂ extract of hop used in the current study contains a number of hop bitter acids (e.g., cohumulone, humulone, adhumulone, colupulone, lupulone, and adlupulone). These α - and β -acids are potent ligands for human T2R (hT2R)–1, 14, and 40, exhibiting reported thresholds of activation as low as 3 nM (48). All 3 hop-responsive hT2Rs have previously been identified in either the small (31) or large intestine (101, 102). However, little is known regarding the profile of hT2R expression in specific EEC cell types. The functional data from the current study would suggest CCK, GLP-1, PYY, and ghrelin-producing EECs express T2R-1, 14, or 40, a T2R expression profile not shared by GIP-producing EECs.

Some limitations of our study should be noted. As a crude extract, the hop treatments contain numerous compounds with the potential to interact with various EEC receptors and signaling pathways other than T2Rs. Other compounds derived from hops acids have previously been examined as antiobesity targets (49, 50, 55, 57), and the potential exists for overlapping or synergistic mechanisms of action. Furthermore, targeted delivery of hop extract to the duodenum may not have occurred in all cases, as the press-fit delayed-release capsules used can leak (103) or disassemble (104) under gastric conditions *in vitro*. In addition, the short intervals between the ad libitum lunch, snack,

and end of daily monitoring may have prevented appropriate treatment differences developing in appetitive VAS measures such as hunger, fullness, and prospective consumption (58). The study could also have benefited from the inclusion of measures of gastric emptying to support this as a mechanism of action. A limitation of the statistical analysis is the increased risk of making a type I error that results from testing multiple outcomes without *P* value adjustment. Finally, the effects of repetitive or chronic administration of hop extract on appetite regulation, including possible compensatory mechanisms and effects on weight management, are unknown. Thus, further long-term studies are warranted.

In conclusion, both gastric and duodenal delivery of a bitter hop extract suppressed EI and modified the release of hormones involved in appetite and glycemic regulation, providing a potential “bitter brake” on EI in healthy-weight males.

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Data Availability

Data described in the manuscript, code book, and analytic code will be made available upon request pending application and approval.

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