Effects of intraduodenal or intragastric administration of a bitter hop extract (*Humulus lupulus* L.), on upper gut motility, gut hormone secretion and energy intake in healthy-weight men

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ABSTRACT

Gastrointestinal functions, particularly pyloric motility and the gut hormones, cholecystokinin and peptide YY, contribute to the regulation of acute energy intake. Bitter tastants modulate these functions, but may, in higher doses, induce GI symptoms. The aim of this study was to evaluate the effects of both dose and delivery location of a bitter hop extract (BHE) on antropyloroduodenal pressures, plasma cholecystokinin and peptide YY, appetite perceptions, gastrointestinal symptoms and energy intake in healthy-weight men. The study consisted of two consecutive parts, with part A including n = 15, and part B n = 11, healthy, lean men (BMI 22.6 ± 1.1 kg/m^2, aged 25 ± 3 years). In randomised, double-blind fashion, participants received in part A, BHE in doses of either 100 mg ("ID-BHE-100") or 250 mg ("ID-BHE-250"), or vehicle (canola oil; "ID-control") intraduodenally, or in part B, 250 mg BHE ("IG-BHE-250") or vehicle ("IG-control") intragastrically. Antropyloroduodenal pressures, hormones, appetite and symptoms were measured for 180 min, energy intake from a standardised buffet-meal was quantified subsequently. ID-BHE-250, but not ID-BHE-100, had modest, and transient, effects to stimulate pyloric pressures during the first 90 min (P < 0.05), and peptide YY from t = 60 min (P < 0.05), but did not affect antral or duodenal pressures, cholecystokinin, appetite, gastrointestinal symptoms or energy intake. IG-BHE-250 had no detectable effects. In conclusion, BHE, when administered intraduodenally, in the selected higher dose, modestly affected some appetite-related gastrointestinal functions, but had no detectable effects when given in the lower dose or intragastrically. Thus, BHE, at none of the doses or routes of administration tested, has appetite- or energy intake-suppressant effects.

1. Introduction

The presence of nutrients in the gastrointestinal (GI) lumen modulates GI motility, particularly the stimulation of pyloric contractions, which act as a brake to regulate flow of chyme from the stomach to the duodenum (Pilichiewicz et al., 2006; Ryan et al., 2012), and, thus, are a key regulator of gastric emptying (Houghton et al., 1988). Small intestinal nutrients also suppress energy intake (Seimon et al., 2010). There is substantial evidence that gut hormones, including cholecystokinin (CCK) and peptide YY (PYY), mediate, at least in part, the effects of

nutrients on GI motility and gastric emptying, as well as energy intake (Steinert et al., 2017), although the latter effect is primarily only apparent when hormones are administered intravenously at relatively high doses (Lim & Poppitt, 2019). There is increasing evidence that non-nutritive bitter compounds may also modulate these GI functions (Rezaie et al., 2021), and, therefore, may have the capacity to modulate energy intake.

In humans, bitter compounds are detected by the taste 2 receptor family of G-protein-coupled receptors, present throughout the GI tract (Rezaie et al., 2021; Steensels & Depoortere, 2018). Stimulation of these

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receptors triggers the release of gut hormones (Chen et al., 2006; Jeon et al., 2011; Kim et al., 2014; Le Neve et al., 2010). For example, the bitter substances, denatonium benzoate, phenylthiocarbamide and steroid glycosides, stimulated CCK from mouse STC-1 or HuTu-80 cells (Chen et al., 2006; Jeon et al., 2011; Le Neve et al., 2010), and denatonium benzoate stimulated PYY from human NCI–H716 cells and mouse duodenal tissue (Kim et al., 2014). Studies in humans have yielded inconsistent and, if any, often modest outcomes (Andreozzi et al., 2015; Bitarafan et al., 2019; van Avesaat et al., 2015). For example, slow intraduodenal infusion of quinine, delivering doses of 37.5–225 mg over 60 min, i.e. in low concentration, did not affect CCK or PYY (Bitarafan et al., 2019; van Avesaat et al., 2015), while intragastric administration of 18 mg quinine in a capsule stimulated CCK modestly (Andreozzi et al., 2015).

The effects of bitter compounds on GI motility have been evaluated in few human studies. In healthy males, quinine, in the dose of 600 mg, administered either intraduodenally 30 min, or intragastrically 60 min, before a nutrient-drink, slowed gastric emptying of the drink, with no difference between the two routes of administration (Rose et al., 2021), indicating the importance of interaction of quinine with small intestinal bitter receptors. Moreover, intragastric quinine (~270 mg), or denatonium benzoate (~30 mg), impaired fundic relaxation and decreased antral, but not duodenal, motility (Avau et al., 2015; Deloose et al., 2017, 2018), while the effect on pyloric pressures is unknown.

Hop flowers, *Humulus lupulus* L., have a long tradition of use as bittering agents (Liu et al., 2015) and contain a range of bitter compounds, including α-acids, β-acids and small amounts of xanthohumol, a prenylated chalconoid (Taniguchi et al., 2015; Zhang et al., 2004). Bitter hop extract (BHE) has been reported to stimulate CCK and PYY release from murine STC-1 cells *in vitro* (Yamazaki et al., 2018) and to reduce hunger, body weight, fat mass and improve glucose homeostasis in rodents (Everard et al., 2012; Kok et al., 2018; Morimoto-Kobayashi et al., 2015; Tripp et al., 2012; Yajima et al., 2004, 2005; Yamazaki et al., 2018) and humans (Morimoto-Kobayashi et al., 2016; Obara et al., 2009; Walker et al., 2019; Yajima et al., 2004). In our recent study, administration of 500 mg of BHE in humans, targeting either the stomach or duodenum using delayed-release capsules, suppressed subsequent *ad libitum* energy intake by ~17%, an effect accompanied by increases in plasma CCK, glucagon-like peptide-1 (GLP-1) and PYY concentrations after both treatments (Walker et al., 2022). However, an observed modest increase in GI 'discomfort' may have been a confounding factor (Walker et al., 2022). The effect of bitter substances on energy intake is not clear-cut, a view supported by a number of recent reviews (Hassan et al., 2023; Klaassen et al., 2021), although these did not consider the effects of bitter hop extracts. It is unknown whether lower doses (e.g. 100 mg or 250 mg, which in a previous study reduced hunger during a 24-h fast (Walker et al., 2019)), would minimise, or eliminate, GI side effects, while retaining the intake-suppressant effect. Interestingly, 'targeted' gastric and duodenal delivery of BHE using capsules had comparable effects (Walker et al., 2022), despite reports that bitter receptors are more abundant in the duodenum (Imai et al., 2020; Wu et al., 2002). It is important to recognise that the timing and the exact amounts of BHE entering the small intestine, and whether they differed sufficiently between the two study days using this approach, is not known. Accordingly, the gastric vs duodenal site of action of BHE remains to be clarified, which can be achieved experimentally by administering it directly to these regions. Whether other mechanisms, e.g. slowing of gastric emptying, played a role, is also unclear.

Therefore, we investigated the dose-related effects of direct intraduodenal or intragastric administration of BHE, in doses of 100 mg and 250 mg, on antropyloroduodenal pressures (which underlie the slowing of gastric emptying), plasma CCK and PYY concentrations, appetite, GI symptoms and energy intake. We performed the study in healthy-weight men to characterise physiological responses in health.

2. Materials and methods

2.1. Participants

Healthy, lean men participated in this study, 15 in part A (age: 24 ± 5 y, body mass index (BMI): 22.9 ± 2 kg/m^2) (Supplemental Fig. 1) and 11 in part B (age: 27 ± 2 y, BMI: 22.3 ± 0.1 kg/m^2) (Supplemental Fig. 2). The study parts were performed consecutively, with different participants in each part, except for five who participated in both. Participants were recruited through advertisements placed online, including the University of Adelaide website and a local advertising website, Gumtree, and flyers placed around local universities and the Royal Adelaide Hospital. Exclusion criteria were being vegetarian or a high-performance athlete, smoking, consumption of >20 g alcohol/day on >5 days/week, history of GI disease or surgery, any food allergy or intolerance, or the use of medications known to affect energy intake, appetite or GI function. All participants were required to be weight-stable (<5% change in body weight) for at least 3 months preceding the study, and unrestrained eaters with a score of ≤12 on the eating-restraint component of the Three-Factor Eating Questionnaire (Stunkard & Messick, 1985). The study protocol was approved by the Human Research Ethics Committee of the Central Adelaide Local Health Network (CALHN reference: R20180631) and the two parts of the study were registered as clinical trials with the Australian New Zealand Clinical Trials Registry (www.anzctr.org.au; part A: ACTRN12619000813189, part B: ACTRN12620000503921). The study was performed in accordance with the Declaration of Helsinki with all participants providing written, informed consent before their inclusion.

Once a participant was enrolled in the study, they were assigned to a treatment order of balanced randomisation that was generated with an online tool (www.randomization.com) by a research officer who was not involved in the data analysis. Both the participant and the investigator who assessed outcomes were blinded to the randomisation.
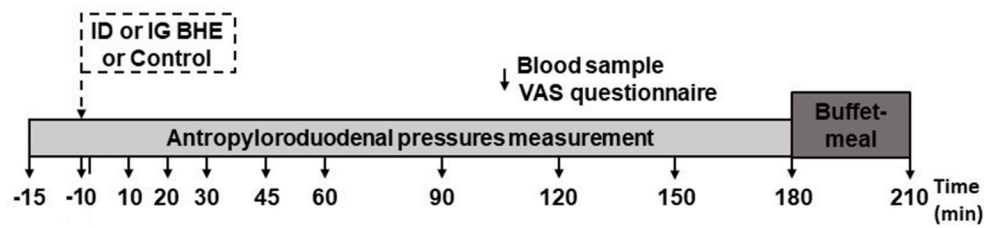
2.2. Study design

Study part A was a 3-arm cross-over that evaluated the effects of intraduodenal (ID) administration of BHE in doses of 100 mg ("ID-BHE-100") or 250 mg ("ID-BHE-250"), or control (canola oil; "ID-control"), on antropyloroduodenal pressures (primary outcome), and plasma CCK and PYY concentrations, appetite perceptions, GI symptoms and energy intake (secondary outcomes), in healthy men. Study part B was a 2-arm cross-over evaluating the effects of intragastric (IG) administration of BHE. Since, in study part A there was no detectable effect of the 100-mg dose (ID-BHE-100), study part B evaluated only the effects of the dose of 250 mg ("IG-BHE-250") and control ("IG-control"). Since IG administration had no effect on antropyloroduodenal pressures (see Results) in part B, GI hormones were not evaluated.

Direct ID or IG administration was used as it enabled standardised administration of BHE to the region of interest and, in the case of ID administration, without the potential cofounding effect of gastric emptying.

2.3. Study treatments

The treatments comprised of 0.6 mL of either (i) 250 mg of a supercritical CO_2 BHE (*Humulus lupulus* L. Pacific Gem™, sourced from New Zealand Hops Ltd, NZ) dissolved in 0.342 mL canola oil (Goodman Fielder, Sydney, New South Wales, Australia), (ii) 100 mg BHE dissolved in 0.497 mL canola oil, or (iii) 0.6 mL canola oil as control. The treatments had comparable densities, i.e. 1.034 ± 0.006 g/mL for the BHE treatments and 0.914 g/mL for canola oil.

All treatments were prepared in the morning of each study day by a research officer who was not involved in the data analysis. Treatments were kept in the dark to minimise photo-oxidation and at a temperature of 30 °C to prevent precipitation of BHE prior to use. The treatments

Fig. 1. Schematic representation of the study design. At t = −10 min, immediately after the occurrence of a phase III, ie, during phase I, of fasting motor activity, with the manometric catheter positioned across the pylorus, a baseline blood sample was taken, and the participant completed a visual analogue scale (VAS) questionnaire for the assessment of appetite perceptions and GI symptoms. At t = −1 min, in part A, bitter hop extract (BHE) in doses of either 100 mg or 250 mg, or control, administered intraduodenally, or in part B, 250 mg BHE or vehicle, was administered intragastrically, within 1 min. Antropyloroduodenal pressures were recorded continuously, and blood samples and VAS questionnaires collected at regular time points. At t = 180 min, the motility recording was terminated and the manometric catheter removed. The participant was then presented with a buffet-style meal and instructed to eat until he was comfortably full. At t = 210 min, another blood sample was collected, and a VAS questionnaire administered, after which the participant was free to leave the laboratory.

were loaded into 1-mL syringes and, although the solutions were comparable in colour, were covered to conceal them from both the study participant and the investigator performing the study. Moreover, the catheter was non-transparent, thus, the solutions could not be seen during administration. The doses were based on a previous study evaluating the effects of BHE on hunger during a 24-h fast (Walker et al., 2019).

2.4. Study protocol

Study visits in each study part were separated by 3–7 days, and performed in randomised, double-blind, cross-over fashion. Participants were instructed to abstain from strenuous exercise and alcohol consumption for 24 h prior to each study visit and provided with a standardised meal (beef lasagne; McCain Food, Wendouree, Victoria, Australia; energy content: 602 kcal) to be consumed (in full and with no additional foods or drinks, except water) by 7 p.m. on the night before each visit.

On the morning of each study day, each participant attended the Clinical Research Facility, Adelaide Medical School, University of Adelaide, at 8 a.m. after an overnight fast from both solids and liquids after 7 p.m. (except water which was allowed until 6.30 a.m.). Upon arrival, an intravenous cannula was placed in the cubital fossa, and the arm was kept warm with a heat pad for regular blood sampling of 'arterialised' blood. The participant was intubated via an anaesthetised nostril with a 17-channel manometric catheter (total length: 100 cm, external diameter: 3.5 mm; Dentsleeve International, Mui Scientific, Mississauga, Ontario, Canada) into the stomach, and the catheter was then allowed to pass into the duodenum by peristalsis. The catheter consisted of six channels positioned in the antrum, a 4.5-cm pyloric sleeve sensor with two channels situated on the back, and seven channels positioned in the duodenum, with all side holes spaced at 1.5-cm intervals (Ryan et al., 2012). A dedicated infusion channel, located ~14.5 cm distal to the pylorus, was used for ID administration (in study part A), and the most proximal antral channel for IG administration (in study part B). The correct positioning of the catheter, with the sleeve sensor straddling the pylorus, was maintained by continuous measurement of the transmucosal potential difference between the most distal antral, and most proximal duodenal, channels (Ryan et al., 2012). Once the catheter was positioned correctly (within 90 ± 13 min), fasting motility was observed until phase III of the interdigestive migrating motor complex occurred, a motor pattern characterised by high-amplitude contractions at the frequency of the intestinal pacemaker and lasting for ~5–10 min. Immediately after the cessation of phase III, during phase I (a period of motor quiescence), at t = −10 min, a baseline blood sample was taken for measurement of plasma CCK and PYY concentrations, the participant completed a visual analogue scale (VAS) questionnaire to assess appetite perceptions and GI symptoms (Parker et al., 2004), and fasting motility was monitored continuously for 10 min (t = -10–0 min) (Fig. 1). Treatments where administered at t =-1 min intraduodenally (study part A), or intragastrically (study part B), within 1 min. Antropyloroduodenal

pressures were then recorded continuously, and blood samples and VAS questionnaires collected at regular time points (t = 10, 20, 30, 45, 60, 90, 120, 150, 180 min) throughout the study. At t = 180 min, the motility recording was terminated and the manometric catheter removed. Participants were then presented with a standardised, cold, buffet-style meal individually in separate rooms and instructed to consume as much, or as little, food as they wished until they felt comfortably full, for up to 30 min (t = 180–210 min) (1, 2). Participants were unaware of the purpose of the meal, i.e. to measure energy intake – they were informed that the aim was to investigate the effect of the meal on subsequent hormone release. No distractions, such as newspapers, laptop computers or mobile phones, were allowed during the meal period, however, during the study period prior to the meal, participants were able to read, use laptop computers or undertake other similar sedentary activities, but were not allowed to sleep. At t = 210 min, after completion of the meal, a final blood sample and VAS ratings were collected, the intravenous cannula was removed, and the participant was allowed to leave the laboratory.

2.5. Measurements

2.5.1. Antropyloroduodenal pressures

Antropyloroduodenal pressures were digitised and recorded using a computer-based system running commercially available software (Solar GI, MMS Database software, version 8.17; Medical Measurement Systems BV, Enschede, The Netherlands) as described previously (Ryan et al., 2012), and stored for subsequent analysis. Data were analysed for the number and amplitude of antral, duodenal pressure waves, and isolated pyloric pressure waves (IPPWs), as well as basal pyloric pressure. Antral and phasic pyloric pressure waves were defined by an amplitude of ≥ 10 mmHg with a minimum interval of 10 s between peaks. Duodenal pressure waves were defined by an amplitude of ≥ 10 mmHg with a minimum of 3 s between peaks. Basal pyloric pressure was calculated by subtracting the mean basal pressure (excluding phasic pressures) recorded at the most distal antral channel from the mean basal pressure recorded at the sleeve (Ryan et al., 2012).

2.5.2. Plasma gut hormone concentrations

Blood samples were collected into ice-chilled ethylenediaminetetraacetic acid-treated tubes. Plasma was separated by centrifugation (3200 rpm, 15 min, 4 °C) within 15 min of collection and stored at −80 °C until assayed.

Plasma CCK-8 concentrations (pmol/L) were measured by radioimmunoassay after ethanol extraction using an adaptation of the method of Santangelo et al. (Santangelo et al., 1998). The minimum detectable concentration was 1 pmol/L. The intra- and inter-assay coefficients of variation were ~16.1% and 15%, respectively.

Plasma total PYY concentrations (pmol/L) were measured by radioimmunoassay using an antiserum (kindly donated by Dr B Otto, Medizinische Klinik, Klinikum Innenstadt, University of Munich, Munich, Germany) against human PYY (1-36) (Sigma-Aldrich, St Louis,

MO) and raised in rabbits (Ryan et al., 2012). This antiserum showed <0.001% cross-reactivity with human pancreatic polypeptide or sulphated CCK-8 and 0.0025% cross-reactivity with human NPY. The minimum detectable concentration was 1.5 pmol/L. Intra- and inter-assay coefficients of variation were both 12.7%.

2.5.3. Appetite perceptions and GI symptoms

Appetite perceptions (i.e. hunger, desire to eat, prospective consumption and fullness) were quantified using validated 100-mm VAS questionnaires (Parker et al., 2004). Nausea and bloating were also assessed. The strength of each perception was rated on a 100-mm horizontal line, where 0 mm was anchored by the statement 'sensation not felt at all' and 100 mm by 'sensation felt the greatest'. Participants were asked to place a vertical stroke at the appropriate point on each 100-mm horizontal line to indicate how they were feeling at that time point. Other perceptions, including happiness and anxiety, were also assessed to distract from the main purpose of the questionnaire, but not evaluated.

2.5.4. Energy intake

Energy intake was assessed using a standardised cold, buffet-style meal (Feltrin et al., 2004; Nair et al., 2009). The meal comprised four slices (~120 g) of whole-meal bread, four slices (~120 g) of white bread, 100 g sliced ham, 100 g sliced chicken, 85 g sliced cheddar cheese, 100 g lettuce, 100 g sliced tomato, 100 g sliced cucumber, 22 g mayonnaise, 20 g margarine, an apple (~170 g), a banana (~190 g), 175 g strawberry yogurt, 100 g chocolate custard, 120 g fruit salad, 375 mL iced coffee, 300 mL orange juice and 600 mL water, and had a total energy content of ~2300 kcal (~27% fat, ~52% carbohydrate and ~21% protein) and weight of ~2924 g. The weight of food and liquids consumed at the buffet meal was obtained by recording the weight of each food item (g) before being offered to the participant and at the end of the meal. Energy intake (kcal) was then calculated using commercially available software (Foodworks 9.0; Xyris Software, Queensland, Australia). The reliability of this meal to accurately measure energy intake on repeated visits has been shown previously (Nair et al., 2009).

2.6. Data and statistical analyses

The number of participants for part A was determined by power calculations based on our previous study (Bitarafan et al., 2019). IPPWs were the primary outcome, hence, used for the power calculation. We calculated that 15 participants would allow detection of a difference of 17.5 (an effect size of 0.78) in the total number of IPPWs at $\alpha = 0.05$, with a power of 80%. The number of participants for part B was determined by power calculations based on the data from the initial six participants. We calculated that with 11 participants, we would be able to detect a difference of 35.8 (an effect size of 0.89) in the total number of IPPWs at $\alpha = 0.05$, with a power of 80%.

Basal pyloric pressure, number and amplitude of IPPWs, antral and duodenal pressure waves, and VAS scores were expressed as change from baseline (i.e., $t = -10$ min) to account for small variations in baseline values. Mean basal pyloric pressure, mean amplitudes of IPPWs, total number and mean amplitudes of antral and duodenal pressures were calculated over the 180-min period after treatment administration. Total numbers and mean amplitudes of antral and duodenal pressure waves were used to calculate motility indices using the following equation: motility index (mmHg) = natural logarithm ([sum of amplitudes × number of pressure waves] + 1) (Camilleri & Malagelada, 1984). The number of IPPWs was quantified in 15-min intervals during the 180-min period. Plasma CCK and PYY concentrations were measured in duplicate and means calculated at each time point, and data expressed as absolute values.

Statistical analyses were performed with SPSS software (version 28.0; SPSS Inc.). Number of IPPWs, plasma CCK and PYY concentrations, and VAS scores were analysed using repeated-measures two-way
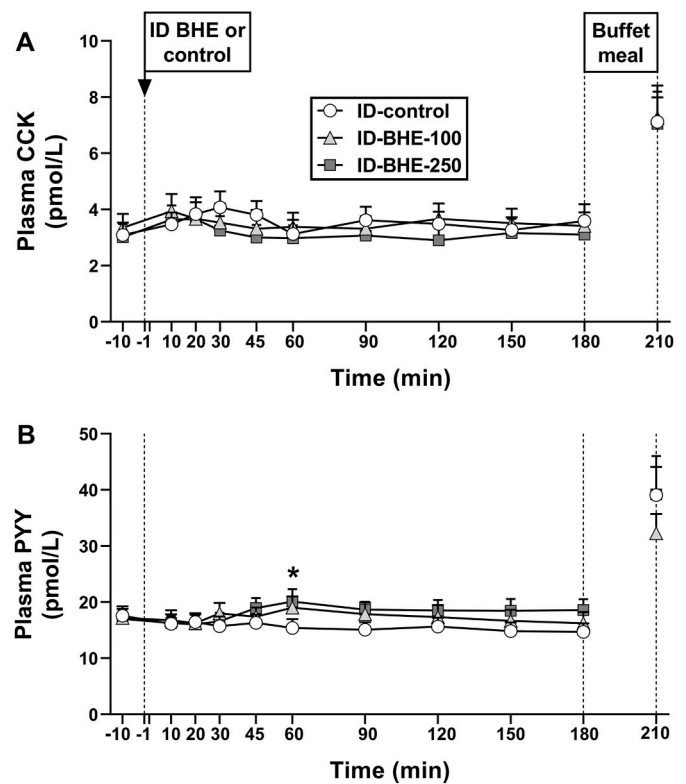
Fig. 2. Plasma cholecystokinin (CCK) (**A**) and peptide YY (PYY) (**B**) concentrations at baseline ($t = -10$ min) and over 180 min ($t = 0$–180 min) after intraduodenal (ID) bolus administration of bitter hop extract (BHE), in the doses of either 100 mg (ID-BHE-100) or 250 mg (ID-BHE-250), or vehicle (canola oil; ID-control), and after the buffet meal, at $t = 210$ min in healthy males. Data were analysed using repeated-measures two-way ANOVA with treatment and time as factors. *Post hoc* comparisons, adjusted for multiple comparisons by Bonferroni's correction, were conducted when ANOVAs revealed significant effects and sphericity of the time effect for all models was evaluated by Mauchly's test and, when violated, the adjusted Greenhouse-Geisser P value was reported. (B) There was a treatment × time interaction for plasma PYY concentrations ($P < 0.01$); ID-BHE-250 stimulated PYY from $t = 60$ min, compared with ID-control (*$P < 0.05$). Data are expressed as means ± SEMs; n = 14 for plasma CCK and n = 15 for plasma PYY.

ANOVA, with within-subject factors of treatment (part A: ID-BHE-100, ID-BHE-250 and ID-control; part B: IG-BHE-250 and IG-control) and time (0–180 min). The number of IPPWs and plasma CCK were also analysed from $t = 0$–90 min in order to capture whether the main effects, if any, occurred primarily 'early'. Total number, mean amplitude and motility indices of antral and duodenal pressures, mean basal pyloric pressure, mean amplitude of IPPWs, energy intake and weight of food (g) consumed from the test meal were analysed using one-way ANOVA with treatment as factor. *Post hoc* comparisons, adjusted for multiple comparisons by Bonferroni's correction, were performed where ANOVAs revealed significant effects and sphericity of the time effect for all models was evaluated by Mauchly's test and, when violated, the adjusted Greenhouse-Geisser P value was reported. All data are reported as means ± SEMs. Differences were considered statistically significant at $P \leq 0.05$.

3. Results

Participants completed all study visits in Part A and B as per protocol and tolerated the study conditions well. No adverse effects were reported. In part A, plasma CCK data were unavailable for one participant owing to assay problems.

Table 1

Effects on antropyloroduodenal pressure waves in response to intraduodenal or intragastric administration of BHE or control in healthy males[a].

	control	BHE-100	BHE-250	ANOVA P value
Part A (ID administration)				
Antral pressure waves				
Total number$_{-10-0\,min}$ (baseline)	1 ± 1	2 ± 1	2 ± 1	0.789
Total number$_{0-180\,min}$	125 ± 23	115 ± 17	132 ± 19	0.615
Mean amplitude$_{0-180\,min}$, mmHg	36 ± 2	33 ± 2	35 ± 2	0.132
Motility index$_{0-180\,min}$, mmHg	12 ± 0	12 ± 0	12 ± 0	0.823
Pyloric pressures				
Mean basal pyloric pressure$_{0-180\,min}$, mmHg	0 ± 0	0 ± 0	0 ± 1	0.596
IPPWs				
Total number$_{-10-0\,min}$ (baseline)	1 ± 0	1 ± 1	0 ± 0	0.233
Total number$_{0-180\,min}$	21 ± 6	29 ± 7	37 ± 8	0.082
Total number$_{0-90\,min}$	8 ± 3	14 ± 4	24 ± 6[#]	0.036
Mean amplitude$_{0-180\,min}$, mmHg	15 ± 2	16 ± 2	16 ± 2	0.663
Duodenal pressure waves				
Total number$_{-10-0\,min}$ (baseline)	20 ± 5	19 ± 6	22 ± 5	0.491
Total number$_{0-180\,min}$	842 ± 86	848 ± 122	921 ± 96	0.273
Mean amplitude$_{0-180\,min}$, mmHg	30 ± 2	26 ± 2	30 ± 2	0.646
Motility index$_{0-180\,min}$, mmHg	16 ± 0	17 ± 0	17 ± 0	0.111
Part B (IG administration)				
Antral pressure waves				
Total number$_{-10-0\,min}$ (baseline)	2 ± 1	–	1 ± 0	0.710
Total number$_{0-180\,min}$	79 ± 11	–	79 ± 19	0.815
Mean amplitude$_{0-180\,min}$, mmHg	53 ± 12	–	55 ± 14	0.507
Motility index$_{0-180\,min}$, mmHg	12 ± 0	–	12 ± 0	0.963
Pyloric pressures				
Mean basal pyloric pressure$_{0-180\,min}$, mmHg	1 ± 2	–	0 ± 1	0.906
IPPWs				
Total number$_{-10-0\,min}$ (baseline)	0 ± 0	–	1 ± 0	0.815
Total number$_{0-180\,min}$	26 ± 5	–	38 ± 11	0.233
Total number$_{0-90\,min}$	15 ± 3	–	24 ± 7	0.247
Mean amplitude$_{0-180\,min}$, mmHg	15 ± 2	–	19 ± 1	0.242
Duodenal pressure waves				
Total number$_{-10-0\,min}$ (baseline)	18 ± 7	–	16 ± 7	0.702
Total number$_{0-180\,min}$	642 ± 106	–	720 ± 70	0.801
Mean amplitude$_{0-180\,min}$, mmHg	26 ± 1	–	27 ± 1	0.525
Motility index$_{0-180\,min}$, mmHg	16 ± 0	–	16 ± 0	0.517

[a] Data are means $\pm$ SEMs; n = 15 in part A (ID administration) and n = 11 in part B (IG administration). BHE-100, bitter hop extract (BHE) in the dose of 100 mg; BHE-250, BHE in the dose of 250 mg; ID, intraduodenal; IG, intragastric; IPPWs, isolated pyloric pressure waves. Data were analysed by one-way ANOVA. *Post hoc* comparisons, adjusted for multiple comparisons by Bonferroni's correction, were performed where ANOVAs revealed significant effects (P ≤ 0.05). #trend for difference from control, P = 0.082.

3.1. Part A: Intraduodenal administration of BHE

3.1.1. Plasma CCK

There were no differences in baseline plasma CCK concentrations between study treatments, and no effects of treatment or time (Fig. 2A).
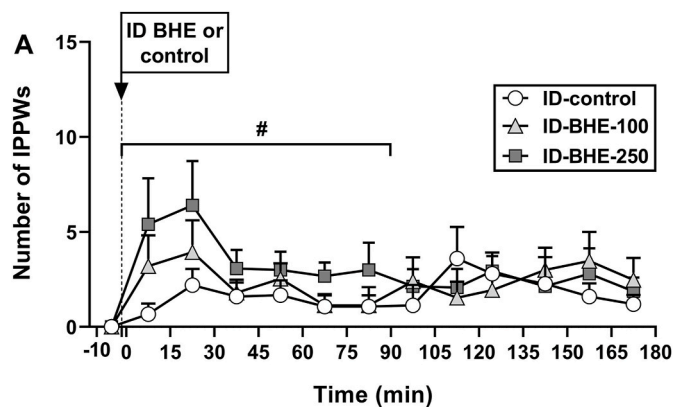
Fig. 3. Number of isolated pyloric pressure waves (IPPWs) during baseline (t = -10–0 min) and over 180 min (t = 0–180 min) after intraduodenal (ID) bolus administration of bitter hop extract (BHE), in the doses of either 100 mg (ID-BHE-100) or 250 mg (ID-BHE-250), or vehicle (canola oil; ID-control) in healthy males. Data were quantified in 10-min and 15-min intervals pre- and post-administration, respectively. Data were expressed as change from baseline and analysed using repeated-measures two-way ANOVA with treatment and time as factors. *Post hoc* comparisons, adjusted for multiple comparisons by Bonferroni's correction, were conducted when ANOVAs revealed significant effects and sphericity of the time effect for all models was evaluated by Mauchly's test and, when violated, the adjusted Greenhouse-Geisser P value was reported. There was an effect of treatment, but not time, on the number of IPPWs during the first 90 min post-administration (P < 0.05); ID-BHE-250 tended to stimulate number of IPPWs, compared with control (#P = 0.082). Data are means $\pm$ SEMs; n = 15.

3.1.2. Plasma PYY

There were no differences in baseline plasma PYY concentrations between study treatments (Fig. 2B). There was a treatment $\times$ time interaction for plasma PYY (P < 0.01); ID-BHE-250 stimulated PYY from t = 60 min, compared with ID-control (P < 0.05) (Fig. 2B).

3.1.3. Antropyloroduodenal pressures

Baseline values for antropyloroduodenal pressures did not differ between study treatments (Table 1). There was a trend for an effect of treatment, but not time, on the number of IPPWs over the 180-min post-administration (P = 0.08), however, *post hoc* tests revealed no differences between treatments. There was an effect of treatment, but not time, on the number of IPPWs during the first 90 min (P < 0.05); ID-BHE-250 tended to stimulate the number of IPPWs, compared with ID-control (P = 0.08) (Fig. 3). There was no effect of treatment on the mean amplitude of IPPWs, mean basal pyloric pressure, or the total number, mean amplitude or motility indices of antral or duodenal pressures (Table 1).
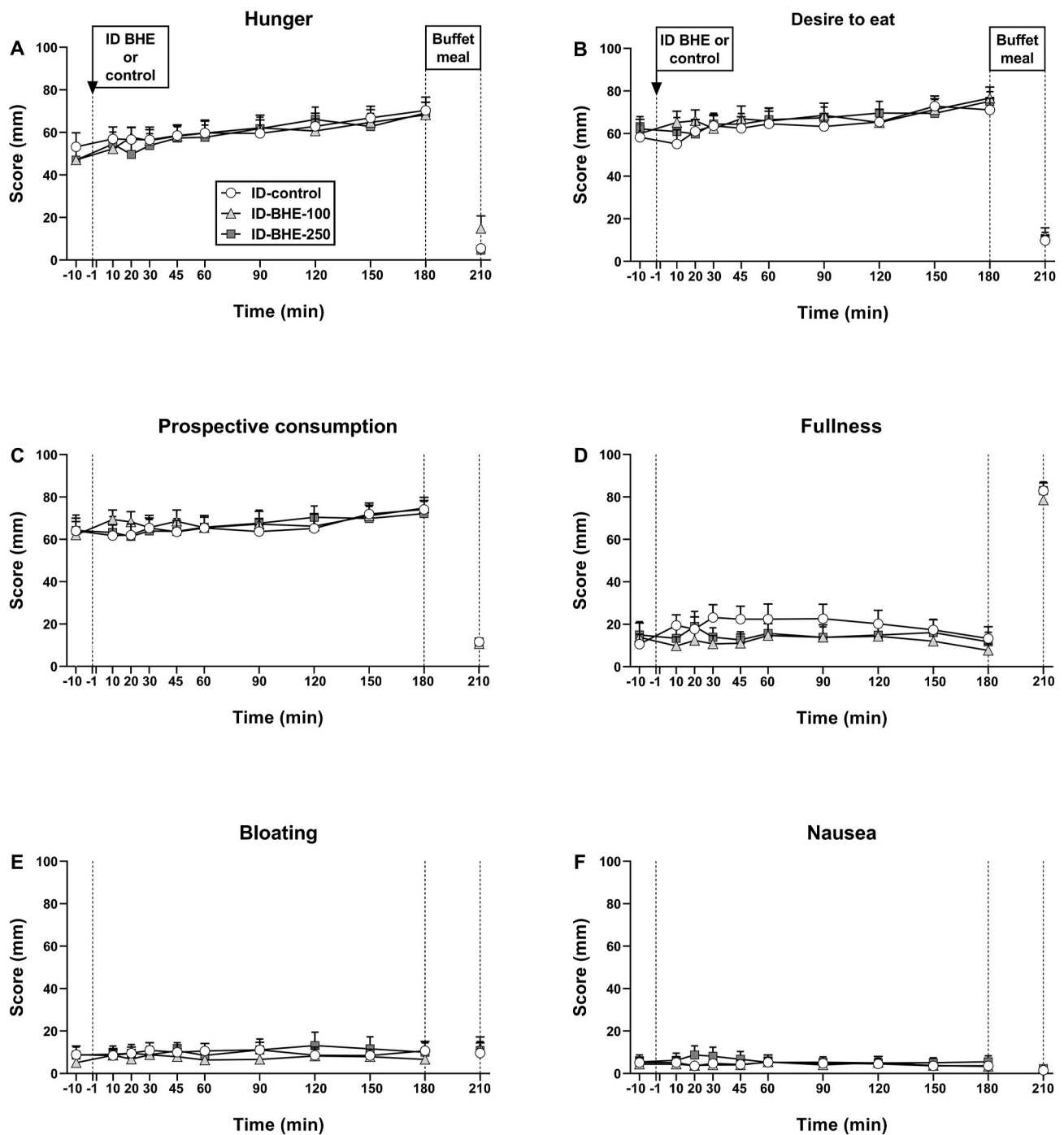
3.1.4. Appetite perceptions, GI symptoms and energy intake

There were no differences in baseline VAS ratings (Fig. 4A–F). There were effects of time, but not treatment, on ratings of hunger, desire to eat and prospective consumption (all P < 0.05), but not fullness. Ratings of hunger and desire to eat increased over time compared with baseline (P < 0.05), while *post hoc* comparisons for prospective consumption were not significant (Fig. 4A–D). There were no effects of treatment or time on ratings of nausea or bloating (Fig. 4E and F).

There were no effects on *ad libitum* energy intake (kcal) or the weight of food consumed (g) from the buffet meal (Table 2).

3.2. Part B: Intragastric administration of BHE

Since, in study part A, there was no detectable effect of the 100-mg dose of BHE, study part B evaluated only the effects of the 250-mg dose. Moreover, because the 250-mg dose had no effect on

Fig. 4. Scores for hunger (**A**), desire to eat (**B**), prospective food consumption (**C**), fullness (**D**), bloating (**E**), and nausea (**F**) at baseline (t = −10 min) and over 180 min (t = 0–180 min) after intraduodenal (ID) bolus administration of bitter hop extract (BHE), in the doses of either 100 mg (ID-BHE-100) or 250 mg (ID-BHE-250), or vehicle (canola oil; ID-control), and after the buffet meal, at t = 210 min in healthy males. Data were analysed using repeated-measures two-way ANOVA with treatment and time as factors. *Post hoc* comparisons, adjusted for multiple comparisons by Bonferroni's correction, were conducted when ANOVAs revealed significant effects and sphericity of the time effect for all models was evaluated by Mauchly's test and, when violated, the adjusted Greenhouse-Geisser P value was reported. Data are expressed as means ± SEMs; n = 15.

antropyloroduodenal pressures (see below), plasma samples were not analysed.

3.2.1. Antropyloroduodenal pressures

Baseline values for antropyloroduodenal pressures did not differ between study treatments (Table 1). There were no effects of treatment, or time, on the number of IPPWs over either 180 min or 90 min post-administration (Fig. 5). There was no effect of treatment on the mean

amplitude of IPPWs, mean basal pyloric pressure, or the total number, mean amplitude or motility indices of antral or duodenal pressures (Table 1).

### 3.2.2. Appetite perceptions, GI symptoms and energy intake

There were no differences in baseline VAS ratings (Fig. 6A–F). There was an effect of time, but not treatment, on ratings of hunger, desire to eat and prospective consumption (all $P < 0.05$), and a trend for an effect

Table 2
Effects on energy content and weight of food consumed at the buffet meal, 180 min after intraduodenal or intragastric administration of BHE or control in healthy males[a].

	control	BHE-100	BHE-250	ANOVA P value
Part A (ID administration)				
Energy intake, kcal	1147 ± 72	1203 ± 93	1141 ± 69	0.358
Weight of food consumed, g	1282 ± 86	1244 ± 73	1293 ± 92	0.606
Part B (IG administration)				
Energy intake, kcal	1133 ± 84	–	1081 ± 98	0.308
Weight of food consumed, g	1181 ± 81	–	1203 ± 104	0.266

[a] Data are means ± SEMs; n = 15 for part A (ID administration) and n = 11 for part B (IG administration). BHE-100, bitter hop extract (BHE) in the dose of 100 mg; BHE-250, BHE in the dose of 250 mg; ID, intraduodenal; IG, intragastric. Data were analysed using one-way ANOVA. *Post hoc* comparisons, adjusted for multiple comparisons by Bonferroni's correction, were performed where ANOVAs revealed significant effects (P ≤ 0.05).
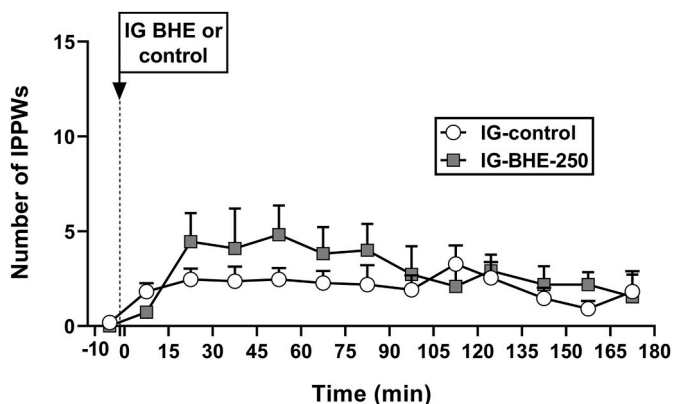
Fig. 5. Number of isolated pyloric pressure waves (IPPWs) during baseline (t = -10–0 min) and over 180 min (t = 0–180 min) after intragastric (IG) bolus administration of either bitter hop extract (BHE), in the dose of 250 mg (IG-BHE-250), or vehicle (canola oil; IG-control) in healthy males. Data were quantified in 10-min and 15-min intervals pre- and post-administration, respectively. Data were expressed as change from baseline and analysed using repeated-measures two-way ANOVA with treatment and time as factors. *Post hoc* comparisons, adjusted for multiple comparisons by Bonferroni's correction, were conducted when ANOVAs revealed significant effects and sphericity of the time effect for all models was evaluated by Mauchly's test and, when violated, the adjusted Greenhouse-Geisser P value was reported. There were no effects of treatment, or time, on the number of IPPWs. Data are means ± SEMs; n = 11.

of time, but not treatment, on ratings of fullness (P = 0.054) (Fig. 6A–D). Ratings for hunger, desire to eat and prospective consumption increased (all P < 0.05), compared with baseline, while *post hoc* comparisons for fullness were not significant. There were no effects of treatment or time on ratings of nausea and bloating (Fig. 6E and F).

There were no effects on *ad libitum* energy intake (kcal) or the weight of food consumed (g) from the buffet meal (Table 2).

4. Discussion

Based on our previous observations of a relatively high dose (500 mg) of BHE, consumed in a capsule, stimulating CCK and PYY and suppressing energy intake, although modest GI discomfort was also reported, the current study evaluated whether lower doses of BHE would also stimulate GI hormones and gut motility, and suppress energy

intake. We found that ID administration of BHE, in the dose of 250 mg, had modest, and transient, effects to stimulate pyloric pressures and plasma PYY, but did not affect plasma CCK, antral or duodenal pressures, appetite perceptions, energy intake, or GI symptoms. Moreover, IG administration of BHE, in the dose of 250 mg, did not appear to have any detectable effects. Taken together, it appears that an intermediate dose of BHE modestly affected some GI functions, but was insufficient to suppress energy intake, and that ID administration is necessary for the observed effects on pyloric pressures and PYY release.

We were particularly interested in the effects of BHE on CCK and PYY, because these hormones have well-established effects to regulate gastric emptying and GI motility and suppress energy intake (Steinert et al., 2017), albeit when administered exogenously rather than through dietary manipulation where outcomes are inconclusive (Lim & Poppitt, 2019). In our recent study, administration of 500 mg BHE in a standard capsule for IG release, or an acid-resistant capsule targeting release to the small intestine, stimulated CCK in response to a subsequent lunch (consumed 60 min and 30 min after intestinal- and gastric-targeted administration, respectively), as well as a snack, consumed 120 min after lunch (Walker et al., 2022). In contrast, in our current study, we found no effect of ID BHE, in the doses of 100 mg and 250 mg, on plasma CCK, suggesting that either these doses were insufficient to trigger CCK release, or that the presence of a meal is required, as in the previous study (Walker et al., 2022), to enhance effects on gut hormone release. In line with the previous findings (Walker et al., 2022), BHE in the 250-mg dose stimulated PYY from ~60 min after administration, although the magnitude of the effect (~3.5 pmol/L) was small, thus, its physiological relevance is unclear. The delay in the effect was likely due to the fact that PYY-secretory cells are predominantly located more distally in the small intestine (Steinert et al., 2017), thus, reflects the time required for BHE to reach that region.

The slowing of gastric emptying induces fullness and contributes to meal termination. The effect of BHE on gastric emptying is currently unknown. We have evaluated the effects of BHE on the motor mechanisms underlying the regulation of gastric emptying, including antral, duodenal and, particularly, pyloric pressures. We found only a modest, and transient, effect of BHE, when administered intraduodenally in the dose of 250 mg, to stimulate pyloric pressures. Since CCK is known to be a key regulator of upper GI motility, particularly pyloric pressures (Brennan et al., 2008; Fraser et al., 1993; Katschinski et al., 1996), the lack of CCK release probably explains the modest effect of BHE to stimulate pyloric pressures. The observed effect may, at least in part, also have been due to a direct effect of BHE on smooth muscle cells, as has been reported for other bitter substances (Avau et al., 2015). In the current study we assume that this was most likely a post-absorptive effect, i.e. BHE accessing the pylorus from the circulation. Whether a higher dose of BHE, which, in the previous study, stimulated CCK (Walker et al., 2022), would stimulate pyloric pressures and be associated with slowing of gastric emptying, and how such effects may relate to the observed suppression of energy intake, warrants further investigation.

Since the primary focus of our study was to evaluate the effects of BHE on GI motility and gut hormones over time, the evaluation of *ad libitum* energy intake, 3 h after BHE administration, was a secondary aim. In a previous study in healthy males, BHE, in doses of 250 mg and 100 mg, consumed twice per day, was reported to reduce hunger during a period of prolonged fasting of up to 20 h, while energy intake was not assessed (Walker et al., 2019). Moreover, in our previous study (Walker et al., 2022), consumption of BHE (500 mg) reduced energy intake from a lunch an hour later by ~277 kcal and from a snack 3 h later by ~225 kcal (Walker et al., 2022). However, the participants also experienced GI symptoms, such as bloating and nausea, which may have, in part, accounted for the suppression of energy intake. It is also known that the longer the interval between a nutrient preload and the subsequent outcome meal (e.g. 180 min vs 90 min vs 30 min), the less the suppression of energy intake (Little et al., 2014). Therefore, the long
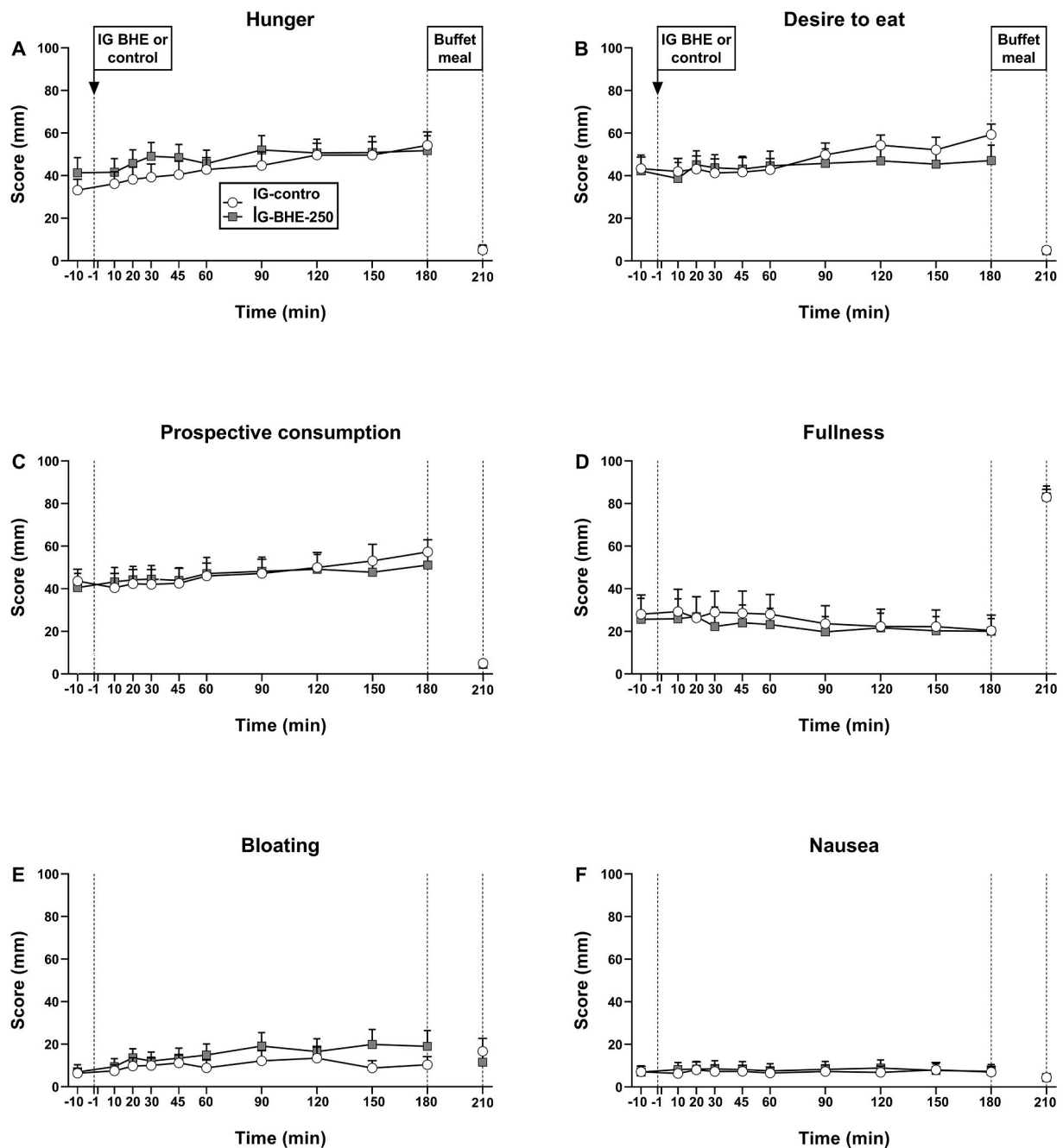
Fig. 6. Scores for hunger (**A**), desire to eat (**B**), prospective food consumption (**C**), fullness (**D**), bloating (**E**), and nausea (**F**) at baseline (t = −10 min) and over 180 min (t = 0–180 min) after intragastric (IG) bolus administration of either bitter hop extract (BHE), in the dose of 250 mg (IG-BHE-250), or vehicle (canola oil; IG-control), and after the buffet meal, at t = 210 min in healthy males. Data were analysed using repeated-measures two-way ANOVA with treatment and time as factors. *Post hoc* comparisons, adjusted for multiple comparisons by Bonferroni's correction, were conducted when ANOVAs revealed significant effects and sphericity of the time effect for all models was evaluated by Mauchly's test and, when violated, the adjusted Greenhouse-Geisser P value was reported. Data are expressed as means ± SEMs; n = 11.

interval between BHE administration and the meal in the current study may also be a reason for the lack of effect on energy intake. The lack of effect on energy intake was unlikely due to intubation with the manometric catheter, as this was tolerated well, i.e. no participants reported any nausea or other discomfort, and in our previous studies, which involved intubation, we have found substantial effects of other treatments to suppress energy intake (Brennan et al., 2008; Little et al., 2014; Ryan et al., 2012).

In contrast to ID BHE, we found no effect of IG BHE on pyloric motility, thus, duodenal exposure appears to be necessary. This is in line with our recent observation that IG administration of quinine (in the

dose of 600 mg, as quinine hydrochloride), did not slow gastric emptying of a mixed-nutrient drink when given 30 min before the drink (Bitarafan et al., 2020), while it significantly slowed gastric emptying when administered either intragastrically 60 min (to allow for greater intestinal exposure), or intraduodenally 30 min, before the drink (Rose et al., 2021). These and our current findings are consistent with observations in animal studies of a greater density of bitter taste receptors in the duodenum than the stomach (Wu et al., 2002). Direct access of IG BHE to gastric smooth muscle cells may also have been hampered by the protective mucus layer. Therefore, effective small intestinal targeting of bitter substances is required to optimise bitter agonist-gut interactions.

Some limitations of our study need to be considered. While we studied males to avoid any confounding effect of the menstrual cycle (Brennan et al., 2009), females have been reported to have lower oral detection thresholds for denatonium benzoate (Deloose et al., 2017) and greater pyloric and glucose-lowering responses to ID than IG quinine (Rezaie et al., 2022), suggesting that they may also be more sensitive to intraluminal BHE. However, we do not believe that the effects of BHE would differ substantially in females. Considering the modest effects of BHE on CCK and PYY observed in part A, other appetite-regulatory gut hormones, e.g. GLP-1, were not analysed. However, based on our previous findings (Feltrin et al., 2004; Ryan et al., 2012) and because GLP-1 and PYY are co-located in enteroendocrine cells (Eissele et al., 1992), we would expect plasma concentrations of GLP-1 to parallel those of PYY. While people with obesity represent the target population for strategies that reduce energy intake, we studied healthy volunteers to establish the magnitude of physiological effects of BHE. In the case of major effects, studies in people with obesity would be pertinent, however, given that the effects were minor, the utility of BHE as a potent appetite-suppressant is questionable.

In conclusion, our study established that, under the experimental conditions of this study, BHE had modest effects when administered intraduodenally, and no effects when administered intragastrically, on some GI functions, including pyloric pressures and plasma PYY, previously found to be associated with the suppression of energy intake. These observations suggest that targeted delivery of BHE into the small intestine is required to induce its effects on these GI functions, while the magnitude of these effects was insufficient to suppress energy intake. Taken together, while BHE has effects to modestly stimulate some appetite-related GI functions, it has no appetite- or energy intake-suppressant effects at any of the administered doses or routes of administration tested.

Author contributions

VB conceived and designed research, performed experiments, analysed the data, interpreted the data, drafted the manuscript, and edited and revised the manuscript; PCEF performed experiments; SDP conceived and designed research and edited and revised manuscript; JRI conceived and designed research and edited and revised the manuscript; CFB conceived and designed research, interpreted the data, drafted the manuscript, edited and revised the manuscript and had primary responsibility for the project. All authors have read and approved the final version of the manuscript.

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Ethical approval

The study protocol was approved by the Human Research Ethics Committee of the Central Adelaide Local Health Network (CALHN reference: R20180631).

Ethical statement

The study protocol was approved by the Human Research Ethics Committee of the Central Adelaide Local Health Network (CALHN reference: R20180631).

Declaration of competing interest

None of the authors have any conflicts of interest to declare.

Data availability

Data will be made available on request.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.appet.2023.106490.

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