INTRAGASTRIC INFUSION OF DENATONIUM BENZOATE ATTENUATES INTERDIGESTIVE GASTRIC MOTILITY AND HUNGER SCORES IN HEALTHY FEMALE VOLUNTEERS

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Abbreviations: CASR: Ca^{2+}-sensing receptor, CCK: cholecystokinin, DB: denatonium benzoate, ERK: extracellular signal-regulated kinase, GIT: gastrointestinal tract, GLP-1: glucagon-like

peptide 1, GPCR: G-protein coupled receptor, HRM: high-resolution manometry, MI: motility index, MMC: migrating motor complex, PROP: *6-n*-propylthiouracil, TAS2R: taste 2 receptor, VAS: visual analogue scale.

ABSTRACT

Background: Denatonium benzoate (DB) has been shown to influence ongoing ingestive behavior and gut peptide secretion.

Objectives: To study the effect of intragastric administration of DB on interdigestive motility, motilin and ghrelin plasma levels, hunger and satiety ratings and food intake in healthy volunteers.

Design: Lingual bitter taste sensitivity was tested using 6 concentrations of DB in 65 subjects. Placebo or DB (1 µmol/kg) were given intragastrically to assess their effect on fasting gastrointestinal motility and hunger ratings; on motilin and ghrelin plasma levels; on satiety and finally on caloric intake.

Results: Women (n=39) were more sensitive towards a lingual bitter stimulus (p=0.005) than men (n=26). In women (n=10), intragastric DB caused a switch in the origin of phase III contractions from stomach to duodenum (p=0.001) and decreased hunger ratings (p=0.04). These effects were not observed in male participants (n=10). In females (n=12), motilin (p=0.04) plasma levels decreased after intragastric DB administration while total and octanoylated ghrelin were not affected. Intragastric administration of DB decreased hunger (p=0.008) and increased satiety ratings (p=0.01) after a meal (500 kcal) in 13 female subjects without affecting gastric emptying in 6 female subjects. Caloric intake tended to decrease after DB administration compared to placebo (720±58 kcal vs 796±45 kcal; p=0.08) in 20 female subjects.

Conclusions: Intragastric DB administration decreases both antral motility and hunger ratings during the fasting state, possibly due to a decrease in motilin release. Moreover, DB decreases hunger and increases satiety ratings after a meal and shows potential to decrease caloric intake. This trial was registered at clinicaltrials.gov as NCT02759926.

Keywords: Bitter, Hunger, Migrating motor complex, Motilin, Denatonium benzoate

Subject group: Nutritional status, dietary intake, and body composition

INTRODUCTION

Denatonium benzoate (DB), benzyl-diethyl (2:6-xylylcarbamoyl methyl) ammonium benzoate, is a strong bitter tastant added to household products to prevent ingestion of potentially harmful substances through taste aversion (1, 2). Concentrations as low as 10 ppb are already detectable, at 50 ppb the taste is distinguishably bitter and at 10 ppm it is described as unpleasantly bitter. In the US, DB is added at a concentration of 6 ppm to denature alcohol (3). Specialized G-protein coupled receptors (GPCRs) from the taste 2 receptor (TAS2R) family are involved in the perception of bitter compounds (4). Until now, 25 TAS2Rs have been identified in humans (5). Based on the sensitivity towards *6-n*-propylthiouracil (PROP), three categories of bitter sensitivity have been identified: non-tasters, medium-tasters and super-tasters (6, 7). It has been reported that more women than men are classified as super-tasters (6, 8).

Besides its extreme bitter taste, DB also has effects on gastrointestinal functions. Direct intraluminal administration of DB in mice has shown to inhibit ongoing ingestive behavior, to suppress food intake and to inhibit gastric emptying (9, 10). Moreover, DB stimulated the *in vitro* release of glucagon-like peptide 1 (GLP-1) and cholecystokinin (CCK), which are known to increase satiety and satiation respectively (11, 12). Intragastric administration of DB in humans has been shown to impair relaxation of the proximal stomach after infusion of a liquid meal and to increase satiation during an oral nutrient tolerance test (13).

During the fasting state, the gastrointestinal tract (GIT) exhibits a specific contractility pattern known as the migrating motor complex (MMC), which can be divided into three phases (14-17). During phase I, no contractions are present in the upper GIT; activity increases during phase II to reach a burst of maximum contractility during phase III, which can originate from

the stomach or the small intestine (14, 15). Exogenous administration of motilin or ghrelin triggers a premature gastric phase III in healthy volunteers (17, 18). Endogenous motilin plasma levels, but not ghrelin, fluctuate in synchrony with antral contractility of the MMC, to reach a peak just before the occurrence of a gastric phase III (16, 17, 19). We recently showed that motilin-induced gastric phase III contractions of the MMC signal hunger in healthy volunteers and that motilin plasma levels were closely associated with interdigestive hunger ratings (20, 21). In 1916, *Carlson* (22) reported an inhibitory effect of intragastrically administered bitter compounds on both fasting gastric contractility and hunger sensations, but the underlying mechanism was not elucidated.

A first objective of the current study was to evaluate gender differences in the bitter taste sensation of orally administered DB in healthy volunteers. Secondly the effect of intragastric administration of DB on hunger ratings and gastrointestinal activity was evaluated. The third aim was to evaluate the role of gastrointestinal hormones in the DB-induced effects. As fourth and fifth objectives, we evaluated if DB was able to attenuate the return of hunger after a standardized meal and to decrease caloric intake respectively.

MATERIAL AND METHODS

This study was approved by the Medical Ethics Committee of the Leuven University Hospital, Leuven, Belgium, and performed in full accordance with the Declaration of Helsinki.

Study design

The current study consisted of 5 independent protocols studying the following parameters: lingual bitter taste sensitivity, gastrointestinal activity, hormonal responses, satiety ratings and food intake.

Test compounds

DB was purchased from Sigma-Aldrich (St Louis, MO, USA). Solutions of DB were prepared in tap water. The stock concentration for intragastric administration was 10 mM. A volume of 0.1 ml/kg bodyweight was administered. The dosage of DB was chosen based on its inhibitory effect on gastric accommodation in healthy volunteers (13). Tap water was given during the placebo condition in a volume of 0.1 ml/kg bodyweight. The pH between the 2 test solutions did not differ (pH7.4).

Subjects

Volunteers were eligible to participate if they were healthy, aged between 18 and 60 years old, had a BMI (in kg/m^2) between 18 and 30, and were recruited from an existing volunteer database in our group. Exclusion criteria were gastrointestinal diseases, abdominal surgery (appendectomy allowed), psychiatric illnesses, and usage of drugs affecting the GIT or central nervous system. Written informed consent was obtained from all volunteers before the start of the study. A total of 65 volunteers (40% men; mean±SEM age: 29±1 y; mean±SEM BMI: 23±0.4) participated in the bitter taste protocol; 20 (50% men; age: 27±9 y; BMI: 24±2)

participated in the gastrointestinal protocol; 12 female subjects (age: 31±4 y; BMI: 22±1) participated in the hormone protocol; 13 female subjects (age: 28±3 y; BMI: 23±1) participated in the satiety protocol and 20 female (age: 23±0.3 y; BMI: 22±1) subjects were included in the food intake protocol. Sample sizes were calculated based on results from previous studies and provided 80% power to detect significant differences of 15% with an alfpha of 0.05 (13, 23, 24). Based on the results obtained from the lingual (study 1) and gastrointestinal (study 2) bitter sensitivity studies it was decided to only include female participants for the last 3 study protocols. A flow chart of the subject distribution can be found in the online supplemental material (**Supplemental Figure 1**). None of the volunteers dropped out. Volunteers that participated in multiple protocols were randomly selected. All subjects were studied after an overnight fast of 12 hours and were asked to refrain from smoking at least 1 hour before the start of the study except for the first study protocol where smoking was not allowed before the start of the study.

Study protocols

Study 1: Bitter taste sensitivity of DB

Six different concentrations (0, 0.1 µM, 1 µM, 10 µM, 0.1 mM and 1 mM) of DB were tested using taste strips (25). The taste strips were placed on the tongue for 90 sec with a closed mouth. Between each concentration participants rinsed their mouth with tap water. The taste strips were given in ascending order of DB concentration, but participants were not aware of this. Bitter taste sensation was scored for each concentration on a 10 cm visual analogue scale (VAS) (0 cm: not bitter at all, 10 cm: extremely bitter).

Study 2: Hunger and gastrointestinal motility responses to intragastric DB administration during the interdigestive state

This study was a placebo-controlled single-blind randomized trial. All these participants also participated in study 1 (Supplemental Figure 1). Placebo (tap water) or DB (1 µmol/kg bodyweight) was administered directly into the upper part of the stomach through a nasogastric feeding tube (Flocare, Nutricia, Bornem, Belgium), 20 min after a complete MMC cycle (**Figure 1A**). The position of the feeding tube was checked with fluoroscopy. By bypassing the tongue, participants could not taste which compound was given. After administration, the measurement continued until the next phase III. Hunger was scored every 5 min on a 10 cm VAS (0 cm: not at all hungry, 10 cm: as hungry as I have ever felt) (26). Adverse events (headache, nausea and stomach ache) were scored every 20 min on a 9-point numerical rating scale.

Study 3: The effect of intragastric DB administration on motilin and ghrelin plasma levels during the interdigestive state

This study was a placebo-controlled single-blind randomized trial. These subjects also participated in study 1 and 10 of them also participated in study 2 (Supplemental Figure 1). Twenty minutes (Figure 1B) after the end of a phase III contraction either placebo (tap water) or DB (1 µmol/kg bodyweight) were administered directly into the upper part of the stomach through a nasogastric feeding tube. The position of the feeding tube was checked with fluoroscopy. The measurement was continued for another 2 hours and blood samples were taken every 10 min to measure motilin and ghrelin plasma levels. The first blood sample was taken 10 min prior to intragastric administration. Hunger was scored every 5 min on a VAS.

Study 4: The effect of DB on hunger and satiety ratings after a meal

This study was a placebo-controlled single-blind randomized trial. Thirty minutes (Figure 1C) after the intragastric administration of placebo (tap water) or DB (1 µmol/kg bodyweight), a

meal, consisting of two pancakes (500 kcal total), was consumed within 15 min. In 6 subjects (31±6 years, 23±2 kg/m²), pancakes ingested on both occasions were labeled with sodium ^{13}C-octanoate. Every 15 min, starting from just before the treatment until 6 hours after the meal, volunteers exhaled in an exetainer that was stored in the fridge for later analysis. The $^{13}CO_2$ secretion data was analyzed by non-linear regression to allow curve fitting and calculation of the gastric half-emptying time. Subjects scored their hunger and satiety feelings prior to the administration and every 15 min for 4 hours starting from the meal intake on a VAS.

Study 5: The effect of DB on food intake

This study was a placebo-controlled double-blind randomized trial. Forty minutes after the intragastric administration of DB (1 µmol/kg bodyweight) or placebo (tap water), subjects ate ad libitum from an excess free-choice buffet for 1 hour. Food items included a variety of pre-sliced, ready-to-eat food items including bread, ham, cheese, lettuce, tomato, mayonnaise, jam, sweets, crisps, rice pudding, waffles, chocolate, apple and banana, which were weighed pre- and postprandially to calculate caloric intake. The total caloric value of the buffet meal was 2330 kcal containing 55 g protein, 94 g fat of which 32 g was saturated and 291 g carbohydrates.

Study techniques

Antroduodenal motility:

Activity of the MMC was measured in study protocol 2 and 3 using a high-resolution solid-state manometry catheter (36 channels, spaced 1 cm apart, Manoscan 360, Sierra Scientific Instruments, Los Angeles, CA, USA, Manoview analysis software v2.0.) as described previously (19). During the manometry measurements, phases of the MMC were identified based on

standardized definitions (27, 28). Motility index (MI) was calculated as follows: (number of contractions*average amplitude contractions*average duration contractions)/5min (19, 29). Average MI was calculated by averaging 6 consecutive antral channels. The anatomical location of the channels was determined via fluoroscopy and through the characteristics of the contractions measured using high-resolution manometry.

Hormone measurements:

Blood samples for motilin detection were collected in lithium heparin tubes containing 500 kIU/ml aprotinin (Roche Applied Science, Penzberg, Germany) and stored at -80°C, after centrifugation, until assayed. Ghrelin blood samples were collected in EDTA tubes supplemented with 500 kIU/ml aprotinin, centrifuged, acidified to a final concentration of 0.1N HCl, extracted on Sep-Pak C18 columns and vacuum-dried. Motilin and ghrelin levels were determined by radioimmunoassay as fully described elsewhere (10, 19).

Statistical analysis

Significance was set at $p<0.05$. BMI and age were compared between sexes using two-tailed unpaired Student's t-tests or Mann Whitney U tests depending on the distribution. Bonferroni correction for multiple testing was applied for post-hoc t-tests. SAS (Statistical Analysis System version 9.3; SAS Institute) was used to analyze the data. Data are represented as mean±SEM or median [Q1, Q3].

Study 1: Bitter taste sensitivity of DB

Bitter taste sensitivity was analyzed using mixed models with BMI, sex, DB concentration and an interaction effect between sex and DB concentration as independent variables. Sex and DB

177 concentration were entered as categorical variables. DB concentration was entered as a
178 repeated within-subject variable.

179 *Study 2: Hunger and gastrointestinal motility responses to intragastric DB administration*
180 *during the interdigestive state*

181 The percentage origin of phase III was compared between placebo and DB with McNemar's
182 test. Paired Student's t-tests were used to compare the interval between administration and
183 phase III contractions between placebo and DB. Percentage change of hunger was calculated
184 with 10 min before intragastric administration as the reference point. Mixed model analysis
185 was used to compare percentage change of hunger between placebo and DB during phase III.
186 Drug (placebo and DB) and time were entered as categorical fixed effects; a drug-by-time
187 interaction effect was included. Drug and time were entered as within-subject variables.

188 *Study 3: The effect of intragastric DB administration on motilin and ghrelin plasma levels*
189 *during the interdigestive state*

190 Mixed model analysis was used to assess the main effects of time and drug (placebo and DB)
191 and the interaction effect between time and drug on the percentage change of hormone
192 plasma levels. Drug and time were entered as within-subject categorical variables. Percentage
193 change of hormone plasma levels was calculated with 10 min prior to intragastric
194 administration as the reference point.

195 The effect of hormone plasma levels on MI antrum was assessed using mixed model analysis
196 with the hormone of interest (motilin, total ghrelin or octanoylated ghrelin), drug (placebo
197 and DB) and time as main effects together with an interaction effect between drug and
198 hormone. Drug and time were entered as categorical within-subject variables. The same

analysis was done for hunger as the dependent variable. Percentage change was used for MI antrum, hormone plasma levels and hunger. The reference point was set at 10 min prior to intragastric administration.

Study 4: The effect of DB on the return of hunger after a meal

Mixed model analysis was used to assess the effect of drug (placebo and DB) on hunger and satiety after a meal intake. Drug and time were included as categorical within-subject main effects together with their interaction effect. Gastric half-time emptying time was compared between the 2 conditions using Wilcoxon signed rank test.

Study 5: The effect of DB on food intake

Our hypothesis for this protocol was that intragastric administration of DB would decrease caloric intake compared to placebo. This hypothesis was formulated based on the results of our previous experiments which are described in the results section (study 2-4). Based on the hypothesized direction of the effect we decided to compare caloric intake between placebo and DB administration using a one-tailed paired Student's t-test.

RESULTS

Women are more sensitive to DB lingual stimulation than men

BMI (p=0.02), but not age (p=0.9), differed between the two sexes (**Table 1**). Increasing concentrations of DB were perceived as more bitter (**Figure 2**; p<0.0001), but a significant sex effect (Figure 2; p=0.005) was also present. There was no interaction effect between sex and DB concentration (p=0.1). There was a trend of a positive association between BMI and bitter taste perception (beta coefficient of 0.3±0.2; p=0.06).

Intragastric administration of DB inhibits gastric phase III and decreases hunger scores in female participants

BMI (p=0.003), but not age (p=0.6), differed between the two sexes (Table 1). None of the volunteers could discriminate between placebo and DB during intragastric administration. No adverse events were reported by any of the participants when DB was given.

In women, administration of DB (**Figure 3**A; p=0.001) reduced the number of phase III contractions with a gastric origin from 67% (placebo) to 33% (DB). The interval between intragastric administration and the occurrence of phase III did not differ (p=0.5) between placebo (76±12 min) and DB (93±12 min) treatment. In men (Figure 3B) there was no (p=0.1) difference in the origin of phase III contractions between placebo (57% gastric) and DB (40% gastric). The interval between intragastric administration and the occurrence of phase III did not differ (p=0.2) between placebo (76±11 min) and DB (111±19 min).

The switch from a gastric to a duodenal phase III origin in females after DB administration was accompanied by a significantly lower percentage change of hunger scores compared to

placebo (Figure 3C; p=0.04). In contrast, in male participants, the percentage change in hunger scores (Figure 3D; p=0.3) during phase III did not differ between placebo and DB treatment.

Intragastric administration of DB inhibits the increase in motilin plasma levels

The effect of intragastric administration of DB on the release of motilin and ghrelin during the interdigestive state was measured. There was a significant main effect of treatment (**Figure 4**A; p=0.04) on the percentage change of motilin plasma levels due to a relative increase in motilin plasma levels during placebo administration and a relative decrease during intragastric administration of DB. There was no difference between the 2 treatment arms for the percentage change of total (Figure 4B; p=0.3) or octanoylated (Figure 4C; p=0.5) ghrelin plasma levels. Values of the raw hormone plasma concentrations can be found in the online supplemental material (**Supplemental Figure 2**).

The change in antral motility was affected by the change in motilin plasma levels (p=0.0003), as well as by DB administration (p=0.02) (**Table 2**). Furthermore, a significant interaction effect between these two factors was found (p=0.01). This interaction effect depicts a significant difference in the slope of the regression curves between placebo and DB. This positive assiciation between antral motility and motilin plasma levels was reduced after DB administration compared to placebo (**Table 3**). A similar result was obtained for the effect of motilin plasma level changes on changes in hunger ratings (**Table 4**). There was a significant main effect of motilin (p=0.0002), DB administration (p=0.02) and a significant interaction effect between the two (p=0.02). The slope of the regression curve between hunger changes and motilin changes differed between placebo and DB (**Table 3**).

Changes in antral motility were not associated with changes in total (p=0.9) or octanoylated ghrelin (p=0.9) (**Table 2**). Changes in total ghrelin plasma levels showed a trend (p=0.06) to be

associated with changes in hunger ratings. There was no associaton between changes in octanoylated ghrelin (p=0.9) plasma levels and changes in hunger ratings (**Table 4**).

Intragastric administration of DB suppresses hunger and increases satiety ratings after a meal

DB administration before the standard meal was associated with prolonged elevated satiety scores and delayed return of hunger after the meal (**Figure 5**). Hunger scores (Figure 5A) were affected by both DB administration (main effect with lower ratings over all time points after DB, p=0.008) and time relative to the meal (p<0.0001). The course of hunger over time tended to differ between placebo and bitter adminsitration (p=0.07). Similarly, satiety scores (Figure 5B) were affected by both DB administration (higher ratings over all time points after DB, p=0.01) and time (p<0.0001). There was no interaction effect between time and bitter administration for satiety scores (p=0.4).

Gastric half-emptying time (measured in 6 subjects) did not differ between placebo and DB (109 [93, 118] min vs 109 [87, 128] min; p=0.7).

Ad libitum food intake tended to decrease after intragastric administration of DB compared with placebo (720±58 kcal vs 796±45 kcal; p=0.08) (**Figure 6**).

DISCUSSION

Our study showed that DB inhibited phase III contractions with gastric origin, with an increased occurrence of phase III starting in the duodenum. In keeping with a role for gastric phase III in determining interdigestive hunger, this switch was accompanied by a decrease in hunger scores (20). Similar to our findings in the lingual system, the response to DB was sex-dependent, being more pronounced in women than in men. The increase in motilin plasma levels was significantly inhibited after DB administration compared to placebo, but ghrelin plasma levels were not affected. The positive association between motilin and antral motility was reduced after intragastric DB administration. A similar result was obtained for the association between motilin and hunger ratings. Moreover, our study showed that intragastric administration of DB decreased hunger and increased satiety scores after a standard meal without altering gastric emptying. Finally, ad libitum food intake tended to decrease after intragastric DB administration.

The most characteristic property of DB is its extreme bitter taste (2). DB is known to interact with 8 of the bitter taste receptors in man (TAS2R4, TAS2R8, TAS2R10, TAS2R13, TAS2R39, TAS2R43, TAS2R46, TAS2R47) (31, 32). Our finding that women perceive a bitter lingual stimulus more intensely than men is in agreement with previous bitter sensitivity studies (6, 8). This sex difference has been associated with the density of fungiform papillae on the anterior tongue and with polymorphisms in the haplotypes of the TAS2R38 gene for PROP sensitivity (6, 7, 33, 34).

In addition we found that in women, but not in men, there was a switch in the origin of phase III contractions from the stomach to the duodenum after administration of DB. This occurred in parallel with a significant inhibition of hunger during phase III. The inhibitory effect of bitter

295 stimuli on antral motility and hunger has already been suggested in the beginning of the
296 previous century by *Carlson* (22). A dose of 1 µmol/kg DB was chosen since this dosage
297 significantly inhibited gastric accommodation without inducing adverse events (13). Also in
298 the present study no adverse events were reported by the volunteers. Two chronic toxicity
299 studies showed no significant changes in general behavior and appearance, ophthalmoscopy,
300 electrocardiograms, body weight, hematological and biochemical studies or urinalysis (35-37).
301 There is only one published case report of adverse reactions due to an allergic reaction after
302 exposure to DB (38). Oral administration of 10 ppm DB to children aged 17-36 months induced
303 a strong taste aversion, but no other effects were noted (1).

304 It needs to be mentioned that the increase in hunger during phase III was weaker in male in
305 comparison to female participants during placebo administration in the current study. One
306 factor contributing to this difference in hunger changes could be the lower occurrence of
307 gastric phase III contractions in male participants during the present study. Previous studies
308 did not report a difference in association of phase III contractions and hunger ratings between
309 men and women (20), but this aspect needs to be studied in more detail and in larger numbers
310 in future research.

311 Our study showed that only motilin plasma levels were decreased after administration of DB.
312 A significant positive association was observed between antral motility and plasma motilin,
313 but not ghrelin levels. This confirms our previous finding that motilin but not ghrelin is the key
314 regulator of the MMC in man (19). After administration of DB, this association between motilin
315 plasma levels and antral motility was reduced, but also the positive relationship between
316 motilin plasma levels and hunger scores, confirming our recently published observation that
317 motilin signals hunger (20).

It has already been described that taste receptors are expressed on enteroendocrine cells, allowing them to modulate the release of several gastrointestinal hormones (11, 12, 39-41). In mice, intragastric administration of DB (10 mM) significantly increased both total and octanoylated ghrelin levels during the first 30 min after gavage (10). Our results in humans differ as we showed no effect of intragastric DB on ghrelin plasma levels. Comparing the two studies is difficult due to species and sex differences and also differences in the dosage. The expression of bitter taste receptors on motilin-producing cells has not been reported but needs to be addressed. In addition, a direct effect of DB on smooth muscle cell contractility cannot be excluded since expression of bitter taste receptors has been shown on human gastric smooth muscle cells. DB administration induced Ca^{2+} rises and increased extracellular signal-regulated kinase (ERK) phosphorylation in human gastric smooth muscle cells (13). Furthermore, bitter compounds induced concentration and region-dependent contractility changes in mouse intestinal muscle strips (13).

However, the current study does not rule out that the findings related to the administration of DB are mediated via a different pathway. *Rogachevskaja et. al.*(42) have shown that DB also binds to the extracellular Ca^{2+}-sensing receptor (CASR). Moreover, this receptor is also expressed in the GIT and has been linked to acid secretion and nutrient sensing (43). Further studies are necessary to elucidate via which pathway DB exerts its effects.

Finally, our study also showed that intragastric administration of DB delayed the return of hunger and prolonged the satiety feeling after a meal without affecting gastric emptying. Intragastric administration of DB in mice was able to delay gastric emptying, but the dosage used was 60 times higher (13). We already reported an effect of DB on satiation during an oral nutrient challenge test and on gastric accommodation in healthy volunteers (13). The effect

of DB on both hunger and satiety could be due to a combined effect on the release of both hunger and satiety hormones. In the present study, we have shown that motilin release is diminished after DB administration which affects hunger scores. Another study performed by *Kim et. al.*(11) showed that administration of DB in mice increased the secretion of GLP-1, a gastrointestinal hormone known to decrease food intake. Moreover it has been reported that intragastric administration of DB in mice activates neurons in the nucleus of the solitary tract possibly via the release of CCK and PYY (44).

Few studies to date have evaluated the effect of bitter agonist administration on food intake in human volunteers. Our results showed a trend that DB administration decreased caloric intake. One study reported that intraduodenal administration of quinine (75 mg in 120 ml tap water) did not alter food intake (45). However, another study reported reduced food intake after intraduodenal administration of quinine (18 mg in an acid-resistant capsule) (23). These differences in outcomes are probably due to differences in compounds, administration routes, dosages and study design. The effect of these compounds on the release of anorexigenic and orexigenic hormones deserves further evaluation in larger subject groups, outside the scope of the present study, as this could lead to the development of new therapeutic approaches in the treatment of obesity.

In summary, for the first time, we provide evidence that DB administered intragastrically is able to decrease both antral motility and hunger during the fasting state. These effects are probably caused by the inhibitory effect of DB on motilin release. Moreover, DB increased satiety and decreased hunger ratings after a standardized meal. These results suggest that DB, and potentially also other bitter tastants, could be investigated for their potential application for the treatment of obesity.

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The authors' responsibilities were as follows:

ED, PJ, MC, JB and IM conducted research; ED, PJ, MC, LVO, ID and JT interpreted and analyzed data; ED wrote the paper; ED, PJ, MC, JB, AR, LVO, ID and JT performed a critical revision of the manuscript; PJ, ID and JT designed the research; JT provided funding. All authors read and approved the final manuscript. None of the authors reported any conflicts of interest.

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TABLES

Table 1. Comparison of BMI and age between male and female participants

	Men	Women	p-value
BMI_study 1 (kg/m²)	24 [21, 26] (n=26)	22 [20, 24] (n=39)	0.02
Age_study 1 (years)	25 [23, 33] (n=26)	26 [23, 32] (n=39)	0.9
BMI_study 2 (kg/m²)	25±2 (n=10)	22±1 (n=10)	0.003
Age_study 2 (years)	23 [22, 26] (n=10)	25 [22, 35] (n=10)	0.6

Data are represented as mean±SEM or median [Q1, Q3]. BMI and age were compared between sexes using two-tailed unpaired Student's t-test or Mann Whitney U test depending on the distribution.

Table 2. The effect of hormone plasma levels on antral motility (n=12)

	Motilin	Total ghrelin	Octanoylated ghrelin
Main effect hormone	0.0003	0.9	0.9
Main effect administration	0.02	0.8	0.2
Main effect time	0.1	0.1	0.1
Hormone by administration	0.01	0.7	0.2

Data were analyzed using mixed model analysis. Antral motility was the dependent variable. The hormone of interest (motilin, total or octanoylated ghrelin), administration, time and an interaction effect between hormone and administration were the independent variables. The table depicts the calculated p-values.

Table 3. The interaction effect between motilin and intragastric administration on antral motility and hunger

	Placebo (n=12)	DB (n=12)
Antral motility	238±46	45±77*
Hunger	6.22±1.2	1.33±2.0*

Values represent betas, which are the slopes of the regression curves. *: $p<0.05$ vs placebo.

Bèta values are given as mean±SEM.

Table 4. The effect of hormone plasma levels on hunger scores (n=12)

	Motilin	Total ghrelin	Octanoylated ghrelin
Main effect hormone	0.0002	0.06	0.9
Main effect administration	0.02	0.3	0.3
Main effect time	0.5	0.6	0.3
Hormone by administration	0.02	0.3	0.4

Data were analyzed using mixed model analysis. Hunger was the dependent variable. The hormone of interest (motilin, total or octanoylated ghrelin), administration, time and an interaction effect between hormone and administration were the independent variables. The table depicts the calculated p-values.

FIGURE LEGENDS

Figure 1. Schematic overview of the protocol outline. All protocols were single-blinded randomized placebo-controlled trials. Either placebo (water) or DB (1 µmol/kg bodyweight) were administered intragastrically using a nasogastric feeding tube. Antroduodenal motility was measured continuously during the course of both study 2 and 3 with high-resolution manometry. Hunger and satiety ratings were scored on 10cm visual analog scales with endpoints. Blood samples were collected via an i.v. catheter and analyzed using hormone-specific RIAs to measure motilin and ghrelin (total and octanoylated) plasma levels. Pancakes were labeled with sodium ^{13}C-octanoate to assess gastric half-emptying time.

Figure 2. Bitter lingual sensitivity in male and female participants. These data are part of study protocol 1. 6 different concentrations of DB were scored for their bitterness using taste strips. Concentrations were presented in ascending order and placed on the tongue for 90 sec. Participants rinsed their mouth between consecutive administrations. Bitter taste perception was scored on a 10-cm visual analog scale. The data was analyzed using mixed-model analysis, with bitter scores as the dependent variable and sex, concentration (repeated statement), BMI and an interaction effect between sex and concentration as the independent variables. Both DB concentration ($p<0.0001$) and sex ($p=0.005$) had a significant effect on bitter taste perception. There was no significant effect of BMI ($p=0.06$) or a significant interaction effect between sex and DB concentration ($p=0.1$). Data are represented as means and SEMs.

Figure 3. Origin of phase III and hunger scores after intragastric administration of DB (1 µmol/kg). These data are part of study protocol 2. Percentage of phase III with gastric and duodenal origin after intragastric administration of placebo or 1 µmol/kg DB in (A) female and (B) male participants. McNemar's test was used to compare the origin between both conditions (*: $p<0.05$). Percentage change in hunger scores during phase III for (C) female and (D) male participants. Percentage change of hunger was calculated with 10 min prior to intragastric administration as the reference point. The data was analyzed with mixed-model analysis. Percentage change of hunger scores was the dependent variable and time, drug and an interaction effect between drug and condition were the independent variables. Both drug and time were entered as repeated categorical variables. In females the percentage change of hunger scores differed significantly between placebo and DB administration ($p=0.04$), there was no significant time ($p=07$) or interaction effect ($p=0.9$). In men there was no significant effect of condition ($p=0.3$) or a significant interaction effect ($p=0.3$), but there was a significant time effect ($p=0.04$). Data (C and D) are represented as means and SEMs.

Figure 4. The effect of intragastric administration of DB on motilin and ghrelin plasma levels.

These data are part of study protocol 3. Percentage change of (A) motilin, (B) total and (C) octanoylated ghrelin plasma levels after intragastric administration of placebo or 1 µmol/kg DB. Time point 0 indicates the start of the intragastric administration. Percentage change of the hormone levels was calculated with 10 min prior to intragastric administration as the reference point. *: $p<0.05$. The data was analyzed with mixed-model analysis. Change in plasma concentration of the hormone of interest was the dependent variable and time, drug and an interaction effect between drug and condition were the independent variables. Both drug and time were entered as repeated categorical variables. Change in motilin plasma levels differed significantly between placebo and DB administration ($p=0.04$) there was no significant time ($p=0.3$) or interaction effect ($p=0.07$). There was no significant main effect of drug administration ($p=0.3$) or time ($p=0.2$) and no significant interaction effect ($p=0.3$) for total ghrelin plasma levels. There was no significant main effect of drug administration ($p=0.5$) or time ($p=0.5$) and no significant interaction effect ($p=0.2$) for octanoylated ghrelin plasma levels. Data are represented as means and SEMs.

Figure 5. The effect of intragastric administration of DB on hunger and satiety scores after a meal. These data are part of study protocol 4. (A) Hunger and (B) satiety scores after placebo or 1 µmol/kg DB intragastric administration. Compounds were administered 30 min before the start of the meal (500 kcal). Time point 0 indicates start of meal intake. *: $p<0.05$ Data was analyzed with mixed-model analysis. Hunger or satiety scores were the dependent variables and time, drug and an interaction effect between drug and condition were the independent variables. Both drug and time were entered as repeated categorical variables. Both hunger and satiety scores were significantly affected by time (both $p<0.0001$) and drug administration ($p=0.008$ and $p=0.01$ respectively). There was no significant interaction effect between time and drug administration for hunger ($p=0.07$) and satiety ($p=0.4$) scores. Data are represented as means and SEMs.

Figure 6. Food intake after intragastric administration of DB. These data are part of study protocol 5. Caloric intake was calculated in kcal. Total caloric value of the excess-choice buffet meal was 4211 kcal. DB (1 µmol/kg) or placebo were given intragastrically 40 min prior to the start of the buffet meal. Subjects had 1 hour to eat ad libitum. Caloric intake between placebo and DB administration was compared using a one-tailed paired Student's t-test (p=0.08).