Intragastric infusion of denatonium benzoate attenuates interdigestive gastric motility and hunger scores in healthy female volunteers[1–3]

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ABSTRACT

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

Objective: We studied how the intragastric administration of DB affects interdigestive motility, motilin and ghrelin plasma concentrations, hunger and satiety ratings, and food intake in healthy volunteers.

Design: Lingual bitter taste sensitivity was tested with the use of 6 concentrations of DB in 65 subjects. A placebo or 1 μmol DB/kg was given intragastrically to assess its effect on fasting gastrointestinal motility and hunger ratings, motilin and ghrelin plasma concentrations, satiety, and caloric intake.

Results: Women (n = 39) were more sensitive toward a lingual bitter stimulus (P = 0.005) than men (n = 26). In women (n = 10), intragastric DB switched the origin of phase III contractions from the stomach to the duodenum (P = 0.001) and decreased hunger ratings (P = 0.04). These effects were not observed in men (n = 10). In women (n = 12), motilin (P = 0.04) plasma concentrations decreased after intragastric DB administration, whereas total and octanoylated ghrelin were not affected. The intragastric administration of DB decreased hunger (P = 0.008) and increased satiety ratings (P = 0.01) after a meal (500 kcal) in 13 women without affecting gastric emptying in 6 women. Caloric intake tended to decrease after DB administration compared with the placebo (mean ± SEM: 720 ± 58 compared with 796 ± 45 kcal; P = 0.08) in 20 women.

Conclusions: Intragastric DB administration decreases both antral motility and hunger ratings during the fasting state, possibly because of a decrease in motilin release. Moreover, DB decreases hunger and increases satiety ratings after a meal and shows potential for decreasing caloric intake. This trial was registered at clinicaltrials.gov as NCT02759926. *Am J Clin Nutr* 2017;105:580–8.

Keywords: bitter, hunger, migrating motor complex, motilin, denatonium benzoate

INTRODUCTION

Denatonium benzoate (DB)[6] [benzyl-diethyl (2:6-xylylcarbamoyl methyl) ammonium benzoate] is a strong bitter tastant added to household products to prevent the ingestion of potentially harmful substances through taste aversion (1, 2). Concentrations ≥10–49 ppb are already detectable, at 50 ppb the taste is distinguishably bitter, and at 10 ppm it is described as unpleasantly bitter. In the United States, DB is added at a concentration of 6 ppm to denature alcohol (3). Specialized G-protein coupled receptors from the taste 2 receptor (TAS2R) family are involved in the perception of bitter compounds (4). A total of 25 TAS2Rs have been identified in humans (5). Based on the sensitivity toward *6-n*-propylthiouracil, 3 categories of bitter sensitivity have been identified: nontasters, medium tasters, and super tasters (6, 7). More women than men have been classified as super tasters (6, 8).

In addition to its extreme bitter taste, DB also affects gastrointestinal functions. The direct intraluminal administration of DB in mice has been shown to inhibit ongoing ingestive behavior, suppress food intake, and inhibit gastric emptying (9, 10). Moreover, DB stimulated the in vitro release of glucagon-like peptide 1 and cholecystokinin, which are known to increase satiety and satiation, respectively (11, 12). The intragastric administration of DB in humans has been shown to impair relaxation of the proximal stomach after the 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 3 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

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[2] The funders had no role in the design, implementation, analysis, or interpretation of the data.

[3] Supplemental Figures 1 and 2 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[6] Abbreviations used: DB, denatonium benzoate; GIT, gastrointestinal tract; MI, motility index; MMC, migrating motor complex; TAS2R, taste 2 receptor; VAS, visual analog scale.

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contractility during phase III that can originate from the stomach or small intestine (14, 15). The exogenous administration of motilin or ghrelin triggers a premature gastric phase III in healthy volunteers (17, 18). Endogenous motilin plasma concentrations, but not ghrelin, fluctuate in synchrony with the 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 concentrations 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.

In this study (NCT02759926), we evaluated *1*) sex differences in the bitter taste sensation of orally administered DB in healthy volunteers, *2*) the effect of intragastric administration of DB on hunger ratings and gastrointestinal activity, *3*) gastrointestinal hormones in the DB-induced effects, *4*) whether DB was able to attenuate the return of hunger after a standardized meal, and *5*) whether DB was able to decrease caloric intake.

METHODS

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

Study design

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

Test compounds

DB was purchased from Sigma-Aldrich. Solutions of DB were prepared in tap water. The stock concentration for intragastric administration was 10 mmol/L. A volume of 0.1 mL/kg body weight was administered. The DB dosage 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 body weight. The pH between the 2 test solutions did not differ (pH 7.4).

Subjects

Volunteers were eligible to participate if they were healthy, aged 18–60 y, had a BMI (in kg/m^2) between 18 and 30, and were recruited from our current volunteer database. Exclusion criteria were gastrointestinal diseases, abdominal surgery (appendectomy allowed), psychiatric illnesses, and the usage of drugs that affect the GIT or central nervous system. Written informed consent was obtained from all volunteers before the start of the study. Data are presented as means ± SEMs. A total of 65 volunteers (40% men; age: 29 ± 1 y; 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 women (age: 31 ± 4 y; BMI: 22 ± 1) participated in the hormone protocol; 13 women (age: 28 ± 3 y; BMI: 23 ± 1) participated in the satiety protocol, and 20 women (age: 23 ± 0.3 y; BMI: 22 ± 1) 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 α of 0.05 (13, 23, 24). Based on the results obtained from the lingual (study 1) and gastrointestinal (study 2) bitter sensitivity studies, we decided to only include women for the last 3 study protocols.

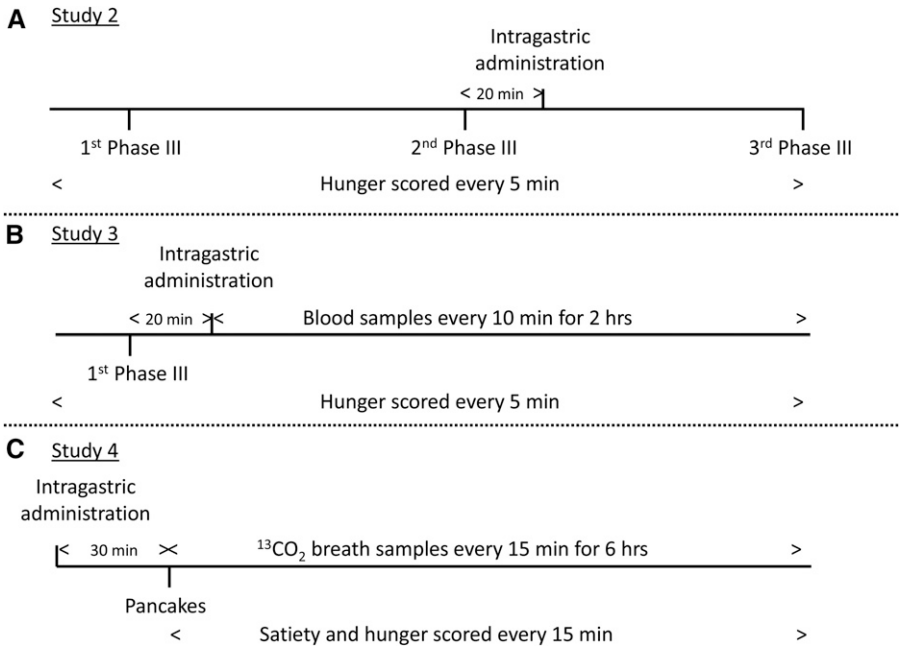

FIGURE 1 Schematic overview of the protocol outline. All protocols were single-blinded, randomized, placebo-controlled trials. Either a placebo (water) or 1 μmol DB/kg body weight was administered intragastrically with the use of a nasogastric feeding tube. Antroduodenal motility was measured continuously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

A flowchart of the subject distribution can be found in **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 h and asked to refrain from smoking ≥1 h before the start of the study except for the first study protocol, in which 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, 1, and 10 μmol/L and 0.1 and 1 mmol/L) of DB were tested with the use of taste strips (25). The taste strips were placed on the tongue for 90 s with a closed mouth. Between each concentration, participants rinsed their mouths with tap water. The taste strips were given in ascending order of DB concentration, but participants were not made aware of this order. Bitter taste sensation was scored for each concentration on a 10-cm visual analog scale (VAS) (0: not bitter at all; 10: 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 participants also participated in study 1 (Supplemental Figure 1). The placebo (tap water) or 1 μmol DB/kg body weight was administered directly into the upper part of the stomach through a Flocare nasogastric feeding tube (Nutricia) 20 min after a complete MMC cycle (**Figure 1**A). The position of the feeding tube was checked with fluoroscopy. By passing 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: "not at all hungry"; 10: "as hungry as I have ever felt") (26). Adverse events (headache, nausea, and stomachache) 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 concentrations 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 the placebo (tap water) or 1 μmol DB/kg body weight was 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 h, and blood samples were taken every 10 min to measure motilin and ghrelin plasma concentrations. The first blood sample was taken 10 min before intragastric administration. Hunger was scored every 5 min on a VAS.

TABLE 1
Comparison of BMI and age between men and women[1]

	Men	Women	*P* value
BMI, kg/m^2			
Study 1	24 (21, 26)[2]	22 (20, 24)	0.02
Study 2	25 ± 2[3]	22 ± 1	0.003
Age, y			
Study 1	25 (23, 33)	26 (23, 32)	0.9
Study 2	23 (22, 26)	25 (22, 35)	0.6

[1] BMI and age were compared between sexes with the use of 2-tailed unpaired Student's *t* tests or Mann-Whitney *U* tests depending on the distribution. Study 1 included 26 men and 39 women. Study 2 included 10 men and 10 women.
[2] Median; IQR in parentheses (all such values).
[3] Mean ± SEM (all such values).

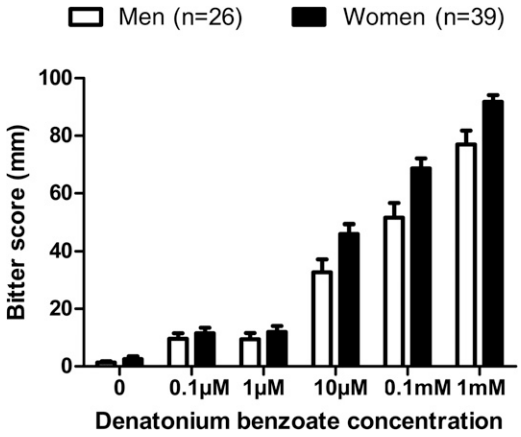

FIGURE 2 Bitter lingual sensitivity in men and women. These data are part of study protocol 1. Six different concentrations of DB were scored for their bitterness with the use of taste strips. Concentrations were presented in ascending order and placed on the tongue for 90 s. Participants rinsed their mouths between consecutive administrations. Bitter taste perception was scored on a 10-cm visual analog scale. The data were analyzed with the use of 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 ($P = 0.06$) nor a significant interaction effect between sex and DB concentration ($P = 0.1$). Data are presented as means and SEMs. DB, denatonium benzoate.

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 the placebo (tap water) or 1 μmol DB/kg body weight, a meal consisting of 2 pancakes (500 kcal) was consumed within 15 min. In 6 subjects (age: 31 ± 6 y; BMI: 23 ± 2), pancakes ingested on both occasions were labeled with sodium [^{13}C]octanoate. Every 15 min, starting from just before the treatment until 6 h after the meal, volunteers exhaled in an exetainer that was stored in a refrigerator for later analysis. The $^{13}CO_2$ secretion data were analyzed with the use of nonlinear regression to allow curve fitting and to calculate the gastric half-emptying time. Subjects scored their level of hunger and satiety before the administration and every 15 min for 4 h 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 the placebo (tap water) or 1 μmol DB/kg body weight, subjects ate from an excess free-choice buffet ad libitum for 1 h. Food items included a variety of presliced ready-to-eat food items, including bread, ham, cheese, lettuce, tomato, mayonnaise, jam, sweets, crisps, rice pudding, waffles, chocolate, apples, and bananas,

which were weighed pre- and postprandially to calculate caloric intake. The total caloric value of the buffet meal was 2330 kcal, and the meal contained 55 g protein, 94 g fat of which 32 g was saturated, and 291 g carbohydrates.

Study techniques

Antroduodenal motility

MMC activity was measured in study protocols 2 and 3 with the use of a Manoscan 360 high-resolution solid-state manometry catheter (Sierra Scientific Instruments) with 36 channels spaced 1 cm apart as described previously (19). During the manometry measurements, MMC phases were identified based on standardized definitions (27, 28). The motility index (MI) was calculated as follows: (number of contractions × mean amplitude contractions × mean duration contractions)/5 min (19, 29). Mean MI was calculated by averaging 6 consecutive antral channels. The anatomic location of the channels was determined with the use of fluoroscopy and through the characteristics of the contractions measured with the use of high-resolution manometry.

Hormone measurements

Blood samples for motilin detection were collected in lithium heparin tubes containing 500 kIU aprotinin/mL (Roche Applied Science). Ghrelin blood samples were collected in EDTA-coated tubes supplemented with 500 kIU aprotinin/mL. Blood samples were centrifuged at 4°C for 10 min at 1278 × *g*. Ghrelin plasma samples were acidified to a final concentration of 0.1 N HCl, extracted on Sep-Pak C18 columns (Waters Corporation, Milford, MA) and vacuum-dried. All plasma samples were stored at −80°C until analysis. Motilin and ghrelin concentrations were determined with the use of radioimmunoassay as described in detail elsewhere (10, 19).

Statistical analysis

Significance was set at $P < 0.05$. BMI and age were compared between sexes with the use of 2-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 version 9.3 (SAS Institute) was used to analyze the data. Data are presented as means ± SEMs or medians (IQRs).

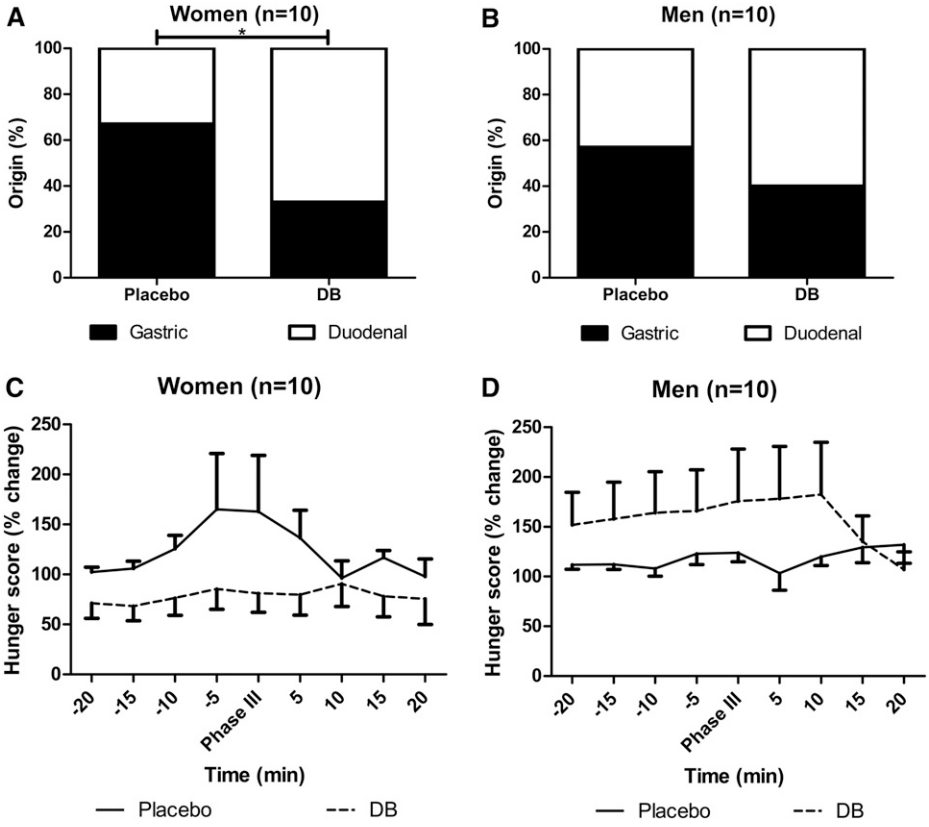

FIGURE 3 Origin of phase III and hunger scores after the intragastric administration of 1 μmol DB/kg. These data are part of study protocol 2. The percentage of phase III with gastric and duodenal origin after intragastric administration of a placebo or 1 μmol DB/kg in women and men are shown in panels A and B, respectively. McNemar's test was used to compare the origin between both conditions ($P < 0.05$). The percentage of change in hunger scores during phase III for women and men is shown in panels C and D, respectively. The percentage of change of hunger was calculated for 10 min before intragastric administration as the reference point. The data were analyzed with mixed-model analysis. The percentage of 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. The percentage of change of hunger scores in women differed significantly between the placebo and DB administration ($P = 0.04$). There was no significant time ($P = 0.07$) or interaction ($P = 0.9$) effect. There was no significant effect of condition in men ($P = 0.3$) or a significant interaction effect ($P = 0.3$), but there was a significant time effect ($P = 0.04$). Data are presented as means and SEMs, $^*P < 0.05$. DB, denatonium benzoate.

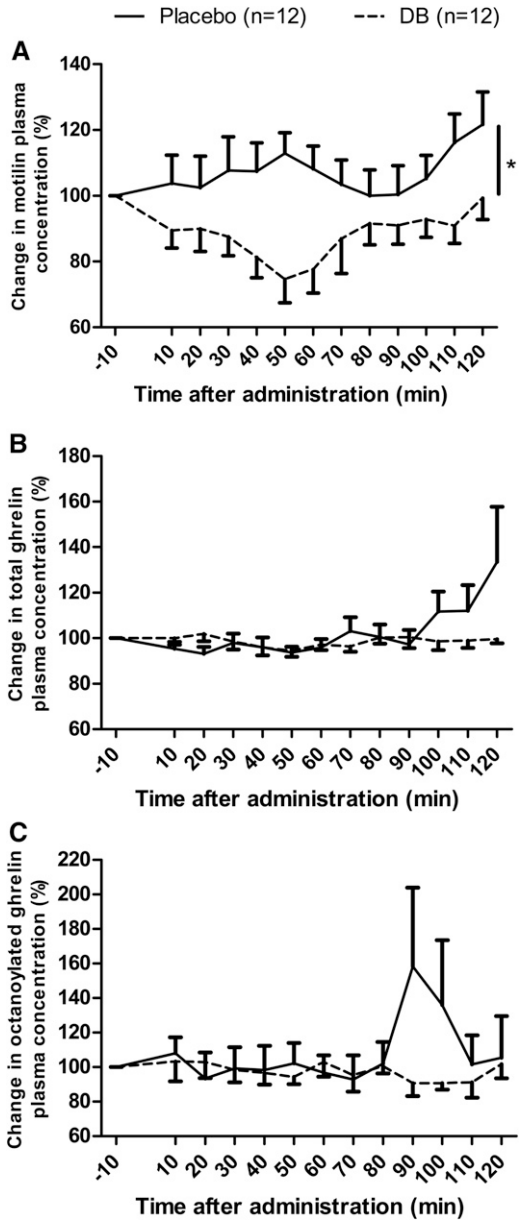

FIGURE 4 The effect of intragastric DB administration on motilin and ghrelin plasma concentrations. These data are part of study protocol 3. The percentage of change of (A) motilin, (B) total, and (C) octanoylated ghrelin plasma concentrations after the intragastric administration of placebo or 1 μmol DB/kg is shown. Time point 0 indicates the start of the intragastric administration. The percentage of change of the hormone concentrations was calculated for 10 min before intragastric administration as the reference point. The data were analyzed with mixed-model analysis. The 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. The change in motilin plasma concentrations differed significantly between the placebo and DB administration ($P = 0.04$). There was no significant time ($P = 0.3$) or interaction ($P = 0.07$) effect. 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 concentrations. 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 concentrations. Data are presented as means and SEMs, $^*P < 0.05$. DB, denatonium benzoate.

Study 1: Bitter taste sensitivity of DB

Bitter taste sensitivity was analyzed with the use of mixed models, with BMI, sex, DB concentration, and an interaction effect between sex and DB concentration as independent variables. Sex and DB concentration were entered as categorical variables. DB concentration was entered as a repeated within-subject variable.

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

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

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

Mixed-model analysis was used to assess the main effects of time and drug (placebo and DB) and the interaction effect between time and drug on the percentage change of hormone plasma concentrations. Drug and time were entered as within-subject categorical variables. The percentage change of hormone plasma concentrations was calculated for 10 min before intragastric administration as the reference point.

The effect of hormone plasma concentrations on the antral MI was assessed with the use of mixed-model analysis, with the hormone of interest (motilin, total ghrelin, or octanoylated ghrelin), drug (placebo and DB), and time as main effects together with an interaction effect between the drug and hormone. Drug and time were entered as categorical within-subject variables. The same analysis was performed for hunger as the dependent variable. The percentage change was used for the antral MI, hormone plasma concentrations, and hunger. The reference point was set at 10 min before intragastric administration.

TABLE 2
Effect of hormone plasma concentrations on antral motility[1]

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

[1] Data were analyzed with the use of mixed-model analysis, $n = 12$. Antral motility was the dependent variable. The hormone of interest, administration, time, and an interaction effect between hormone and administration were the independent variables.

TABLE 3
Interaction effect between motilin and intragastric administration on antral motility and hunger[1]

	β values	
	Placebo	DB
Antral motility	238 ± 46	45 ± 77*
Hunger	6.22 ± 1.2	1.33 ± 2.0*

[1] Values are means ± SEMs. $n = 12$. *$P < 0.05$ compared with placebo. DB, denatonium benzoate.

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

Mixed-model analysis was used to assess the effect of the drug (placebo and DB) on hunger and satiety after a meal. 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 with the use of Wilcoxon's Signed Rank test.

Study 5: The effect of DB on food intake

We hypothesized that the intragastric administration of DB would decrease caloric intake compared with the placebo for this protocol. This hypothesis was formulated based on the results of our previous experiments, which are described under Results (studies 2–4). Based on the hypothesized direction of the effect, we decided to compare caloric intake between the placebo and DB administration with the use of a 1-tailed paired Student's *t* test.

RESULTS

Women are more sensitive than men to DB lingual stimulation

BMI ($P = 0.02$), but not age ($P = 0.9$), differed between the 2 sexes (**Table 1**). Increasing concentrations of DB were perceived as more bitter ($P < 0.0001$) (**Figure 2**), but a significant sex effect ($P = 0.005$) (Figure 2) 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 (β: 0.3 ± 0.2; $P = 0.06$).

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

BMI ($P = 0.003$), but not age ($P = 0.6$), differed between the 2 sexes (Table 1). None of the volunteers could discriminate between the placebo and DB during intragastric administration. No adverse events were reported by any of the participants when DB was given.

In women, DB administration ($P = 0.001$) (**Figure 3**A) reduced the number of phase III contractions, with a gastric origin from 67% (placebo) to 33% (DB). The interval between the intragastric administration and occurrence of phase III did not differ ($P = 0.5$) between the 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 the placebo (57% gastric) and DB (40% gastric). The interval between the intragastric administration and occurrence of phase III did not differ ($P = 0.2$) between the placebo (76 ± 11 min) and DB (111 ± 19 min).

The switch from a gastric to duodenal phase III origin in women after DB administration was accompanied by a significantly lower percentage change of hunger scores than the placebo ($P = 0.04$) (Figure 3C). In contrast, for men, the percentage change in hunger scores ($P = 0.3$) (Figure 3D) during phase III did not differ between the placebo and DB treatment.

Intragastric administration of DB inhibits the increase in motilin plasma concentrations

We measured how the intragastric administration of DB affects motilin and ghrelin release during the interdigestive state. There was a significant main effect of treatment ($P = 0.04$) (**Figure 4**A) on the percentage change of motilin plasma concentrations caused by a relative increase in motilin plasma concentrations during the placebo administration and a relative decrease during the intragastric administration of DB. There was no difference between the 2 treatment arms for the percentage change of total ($P = 0.3$) (Figure 4B) or octanoylated ($P = 0.5$) (Figure 4C) ghrelin plasma concentrations. Values of the raw hormone plasma concentrations can be found in **Supplemental Figure 2**.

The change in antral motility was affected by the change in motilin plasma concentrations ($P = 0.0003$), as well as by DB administration ($P = 0.02$) (**Table 2**). Furthermore, a significant interaction effect between these 2 factors was found ($P = 0.01$). This interaction effect depicts a significant difference in the slope of the regression curves between the placebo and DB. This positive association between antral motility and motilin plasma concentrations was reduced after DB administration compared with the placebo (**Table 3**). A similar result was obtained for the effect of motilin plasma concentration changes on changes in hunger ratings (**Table 4**). There was a significant main effect of motilin ($P = 0.0002$) and DB administration ($P = 0.02$) as well as a significant interaction effect between them ($P = 0.02$). The slope of the regression curve between hunger changes and motilin changes differed between the 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 concentrations showed a trend ($P = 0.06$) to be associated with changes in hunger ratings. There was no association between changes in octanoylated ghrelin ($P = 0.9$) plasma concentrations and changes in hunger ratings (Table 4).

TABLE 4
Effect of hormone plasma concentrations on hunger scores[1]

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

[1] Data were analyzed with the use of mixed-model analysis. $n = 12$. Hunger was the dependent variable. The hormone of interest, administration, time, and an interaction effect between hormone and administration were the independent variables.

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 a 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 the placebo and bitter administration ($P = 0.07$). 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 the placebo and DB [both 109 min (placebo IQR: 93, 118; DB IQR: 87, 128 ($P = 0.7$)]. Ad libitum food intake tended to decrease after the intragastric administration of DB compared with placebo (720 ± 58 compared with 796 ± 45 kcal; $P = 0.08$) (**Figure 6**).

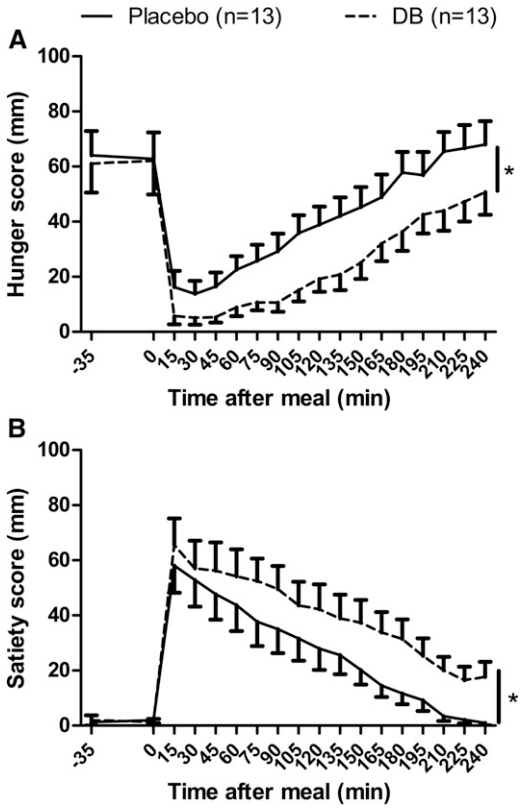

FIGURE 5 The effect of intragastric DB administration on hunger and satiety scores after a meal. These data are part of study protocol 4. Hunger (A) and satiety (B) scores after a placebo or 1 μmol DB/kg intragastric administration are shown. Compounds were administered 30 min before the start of the meal (500 kcal). Time point 0 indicates the start of meal intake. Data were 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 presented as means and SEMs, $^*P < 0.05$. DB, denatonium benzoate.

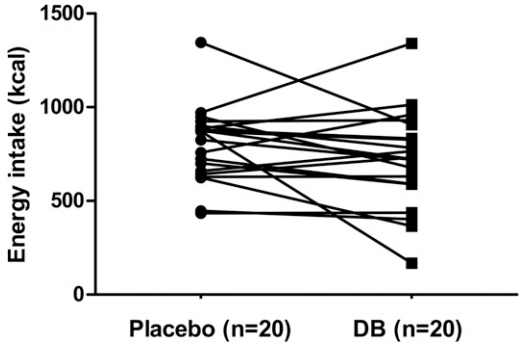

FIGURE 6 Food intake after intragastric DB administration. These data are part of study protocol 5. The total caloric value of the excess-choice buffet meal was 2330 kcal. DB (1 μmol/kg) or a placebo was given intragastrically 40 min before the start of the buffet meal. Subjects had 1 h to eat ad libitum. Caloric intake between the placebo and DB administration was compared with the use of a 1-tailed paired Student's t test ($P = 0.08$). DB, denatonium benzoate.

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 and more pronounced in women than in men. The increase in motilin plasma concentrations was significantly inhibited after DB administration compared with the placebo, but ghrelin plasma concentrations 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 the 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 humans (TAS2R4, TAS2R8, TAS2R10, TAS2R13, TAS2R39, TAS2R43, TAS2R46, and TAS2R47) (30, 31). 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 *6-n*-propylthiouracil sensitivity (6, 7, 32, 33).

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 the administration of DB. This occurred in parallel with a significant inhibition of hunger during phase III. The inhibitory effect of bitter stimuli on antral motility and hunger was suggested by Carlson (22). A dose of 1 μmol DB/kg was chosen because this dosage significantly inhibited gastric accommodation without inducing adverse events (13). In addition, no adverse events were reported by the volunteers in our study. Two chronic toxicity studies showed no significant changes in general behavior and appearance, ophthalmoscopy, electrocardiograms, body weight, hematologic and biochemical studies, or urinalysis (34–36). There is only one published case

report, to our knowledge, of adverse reactions caused by an allergic reaction after exposure to DB (37). Orally administering 10 ppm DB to children aged 17–36 mo induced a strong taste aversion, but no other effects were noted (1).

It needs to be mentioned that the increase in hunger during phase III was weaker in men than in women during the placebo administration in this study. One factor contributing to this difference in hunger changes could be the lower occurrence of gastric phase III contractions in men. Previous studies have not reported a difference in the association of phase III contractions and hunger ratings between men and women (20), but this aspect needs to be studied in more detail and in larger numbers in the future.

Our study showed that only motilin plasma concentrations decreased after DB administration. A significant positive association was observed between antral motility and plasma motilin but not ghrelin concentrations. This confirms our previous finding that motilin but not ghrelin is the key regulator of the MMC in humans (19). After the administration of DB, this association between motilin plasma concentrations and antral motility was reduced, as was the positive relation between motilin plasma concentrations and hunger scores, confirming our recently published observation that motilin signals hunger (20).

It has already been described that taste receptors are expressed in enteroendocrine cells, allowing them to modulate the release of several gastrointestinal hormones (11, 12, 38–40). In mice, the intragastric administration of 10 mmol DB/L significantly increased both total and octanoylated ghrelin concentrations during the first 30 min after gavage (10). Our results in humans differ because we showed no effect of intragastric DB on ghrelin plasma concentrations. Comparing the 2 studies is difficult because of species and sex differences and because of differences in the dosage. The expression of bitter taste receptors on motilin-producing cells has not been examined to our knowledge and needs to be addressed. In addition, a direct effect of DB on smooth muscle cell contractility cannot be excluded because the expression of bitter taste receptors has been shown in human gastric smooth muscle cells. DB administration increased Ca^{2+} and extracellular signal-regulated kinase phosphorylation in human gastric smooth muscle cells (13). Furthermore, bitter compounds induced concentration- and region-dependent contractility changes in muscle strips from the intestines of mice (13).

However, our study does not rule out that the findings related to the administration of DB are mediated via a different pathway. Rogachevskaja et al. (41) showed that DB also binds to the extracellular Ca^{2+}-sensing receptor. Moreover, this receptor is also expressed in the GIT and has been linked to acid secretion and nutrient sensing (42). Further studies are necessary to elucidate the pathway through which DB exerts its effects.

Finally, our study also showed that the intragastric administration of DB delayed the return of hunger and prolonged satiety after a meal without affecting gastric emptying. The intragastric administration of DB in mice was able to delay gastric emptying, but the dosage used was 60 times higher (13). We have 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 caused by a combined effect on the release of both hunger and satiety hormones. In this study, we have shown that motilin release is diminished after DB administration, thus affecting hunger scores. Another study performed by Kim et al. (11) showed that DB administration in mice increased the secretion of glucagon-like peptide 1, a gastrointestinal hormone known to decrease food intake. Moreover, it has been reported that the intragastric administration of DB in mice activates neurons in the nucleus of the solitary tract possibly via the release of cholecystokinin and peptide YY (43).

Few studies to date to our knowledge have evaluated the effect of bitter agonist administration on food intake in humans. Our results showed a trend that DB administration decreased caloric intake. One study (44) reported that the intraduodenal administration of quinine (75 mg in 120 mL tap water) did not alter food intake. However, another study (23) reported reduced food intake after the intraduodenal administration of quinine (18 mg in an acid-resistant capsule). These differences probably resulted from 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 because this could lead to the development of new therapeutic approaches in the treatment of obesity.

In summary, we provide evidence, for the first time to our knowledge, that DB administered intragastrically can 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 other bitter tastants could be investigated for their potential application in treating obesity.

The authors' responsibilities were as follows—ED, PJ, MC, JB, and IM: conducted the research; ED, PJ, MC, LVO, ID, and JT: interpreted and analyzed the data; ED: wrote the manuscript; ED, PJ, MC, JB, AR, LVO, ID, and JT: critically revised the manuscript; PJ, ID, and JT: designed the research; and all authors: read and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

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