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## Appetite suppression through delayed fat digestion

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### Abstract

High-fat diets are often associated with greater caloric intake and weight gain. Since satiety during fat intake is induced by fat in the intestine we investigated the efficiency of a lipid compound that retards fat digestion to regulate fat intake. We found this compound to reduce high-fat food intake, body weight and blood lipids in Sprague–Dawley rats, without causing steatorrhea. The absence of steatorrhea is explained by an increased pancreatic lipase/colipase secretion, compensating the impaired lipolysis by the added compound. The animals also had an elevated CCK secretion. The satiety for fat may be the consequence of elevated CCK and procolipase/enterostatin levels. We conclude that compounds can be found that delay intestinal fat digestion and control high-fat food intake through the release of satiety signals, without causing steatorrhea. The absence of steatorrhea makes such compounds advantageous over lipase inhibitors in the treatment of obesity.

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**Keywords:** Satiety; High-fat food intake; Blood lipids; Fecal fat; Lipase inhibitor; CCK; Enterostatin

### 1. Introduction

A high-fat intake has been suggested to play a crucial role in causing overeating and overweight due to its palatability and greater caloric density [1]. High-fat intake has also been demonstrated to induce diabetes through the lipotoxic effects of free fatty acids on the islets of Langerhans [2]. It is therefore of great importance to restrict fat intake in order to prevent the development of obesity and obesity related disorders.

Regulation of fat intake occurs through a number of peptides. For example, galanin stimulates fat intake [3], while enterostatin [4] inhibits fat intake. PYY has been shown to reduce food intake in high-fat fed rodents and also elevate fat oxidation [5], while CCK has been demonstrated to suppress food intake in rats fed a high-fat diet with an increased potency for a high-fat diet containing 60% fat compared with a diet containing 34% fat [6].

CCK has also been successfully used to diminish appetite in humans [7]. Since satiety for fat is strongly related to the presence of fatty acids formed in the intestine during lipid breakdown, the lipolytic enzymes and their activity in the intestine is of critical importance [8]. Intestinal fat digestion involves several enzymatic systems, the most important being the pancreatic lipase–colipase system [9,10]. The lipase inhibitor Orlistat is in current use for reducing fat intake and body weight [11]. The drawback with Orlistat is the steatorrhea associated with high-fat diets, which results in a low adherence to treatment [12].

In this report we have studied the effect of a compound that retards fat digestion through inhibition of the lipase–colipase mediated triglyceride breakdown on appetite regulation. We found no steatorrhea associated with this compound.

### 2. Materials and methods

#### 2.1. Experimental procedures

Female Sprague–Dawley rats (200 g) from B&K, Sollentuna, Sweden were housed in a temperature-controlled room (22 ± 1 °C) under a 12-h light/12-h dark cycle (lights on at 6 am). All

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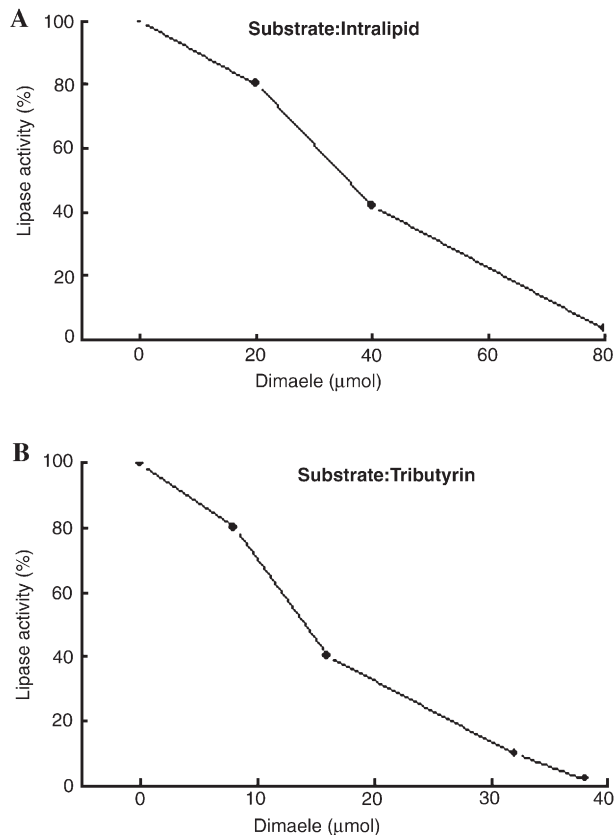


Fig. 1. Inhibition of colipase-dependent lipase activity in vitro by dimaele, using two different triglyceride substrates, Intralipid (A) and tributyrin (B), both substrates dispersed in bile salt. Measurements of lipase activity were carried out using purified lipase and colipase with and without increasing amounts of dimaele, the released fatty acids being continuously titrated in a pH stat.

rats had free access to water and were fed standard rat chow (R36, Lactamin, Kimsta, Sweden) *ad libitum* unless otherwise stated. All procedures were approved by the Local Animal Ethics Committee Lund, Lund, Sweden. Dimethylaminoethyl-dodecylether (dimaele) was synthesised as previously described [13] and dissolved in 1% methylcellulose (long-term study) or in Intralipid (short-term study). Fresh solutions of dimaele were made daily.

## 2.2. Feeding protocol

### 2.2.1. Dose–response effect of dimaele

Thirty-six rats were individually housed in Macrolon cages and were given a high-fat diet *ad libitum* for 1 week prior to the start of the study. The high-fat diet, described previously [14] consisted of 23.9% protein by energy, 34.0% carbohydrate and 42.1% fat by energy with a caloric density of 4.7 kcal/g. After 1 week of feeding the rats were divided into three groups; one group received 338 μmol (37.5 mg) dimaele dissolved in 1 ml 1% methylcellulose, a second group received 445 μmol (50 mg) dimaele dissolved in 1 ml 1% methylcellulose, and the third group 1 ml 1% methylcellulose (control). Dimaele was administered daily through oral gavage at the onset of dark

(6 pm) for six consecutive days. Daily food intake was measured 15 h after dimaele administration from pre-weighed portions. All cages were carefully monitored for evidence of food spillage.

In another set of experiments 36 rats (200 g) were fed a low-fat diet (standard rat chow; R36, Lactamin, Kimsta, Sweden) and divided into three groups, one receiving 338 μmol (37.5 mg) dimaele, a second receiving 445 μmol of dimaele and the

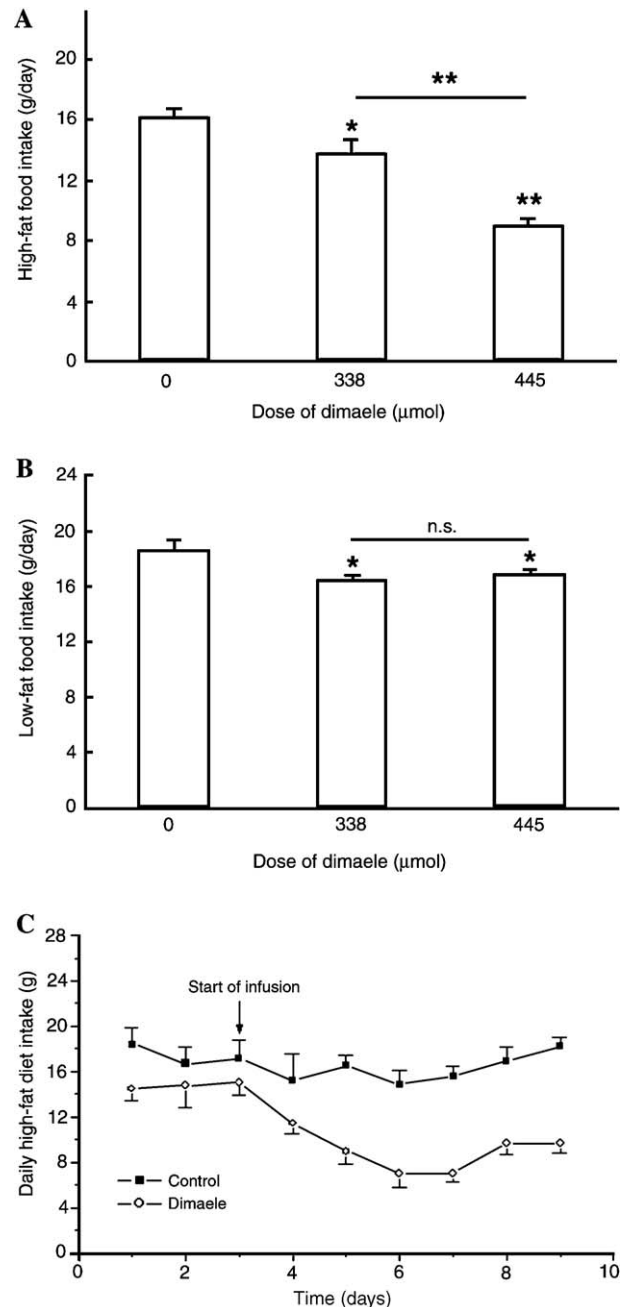


Fig. 2. Effect of dimaele on food intake using either high-fat diet or low-fat diet. Dimaele (338 or 445 μmol) dissolved in 1% methylcellulose was administered once a day through oral gavage at 6 pm prior to feeding either high-fat diet (A) or low-fat diet (B) during 6 days; control rats received 1% methylcellulose. The time course for the effect of dimaele (445 μmol) on food intake during high-fat feeding (C).

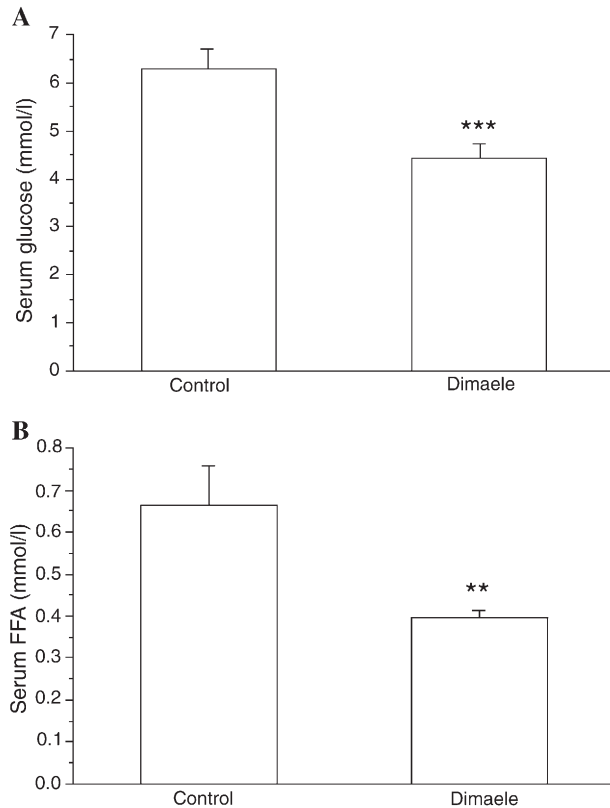


Fig. 3. Serum glucose levels (A) were reduced in rats fasted for 14 h and treated with dimaele during high-fat diet. Dimaele also decreased serum free fatty acids (FFA) levels compared to the control rats (B).

third group serving as control (1% methylcellulose). The procedure was similar as described above.

### 2.2.2. Long-term study on the effect of dimaele

In a long-term study 24 rats fed a high-fat diet were divided into two groups; one group received 445  $\mu$ mol dimaele dissolved in 1 ml 1% methylcellulose, and the second group served as control (1 ml 1% methylcellulose). The dose of dimaele was chosen 10 times its complete inhibition of lipase/colipase with tributyrin as substrate. Dimaele and methylcellulose were administered daily for 6 days by oral gavage. Food intake and body weight was recorded daily. At the end of the experiment blood samples were taken for analysis of blood glucose and serum free fatty acids.

### 2.2.3. Short-term study on the effect of dimaele

In a short-term experiment, 12 rats were fasted for 12 h and divided into two groups; one group was given 1 ml Intralipid (200 mg/ml) (Biovitrum, Sweden) through oral gavage, and the second group received 445  $\mu$ mol (50 mg) dimaele dissolved in 1 ml Intralipid (200 mg/ml). The rats were fasted and blood collected 12 h after the administration of the substances. The rats were anesthetized by intraperitoneal injection of ketamine (ketalar; 100 mg/kg) and xylazine (Rompun; 20 mg/kg). A midline incision was made and the intestinal content collected through pumping 1 ml saline into the intestine at the level of the

duodenum to the jejunum. Intestinal juice was collected and stored at  $-20^{\circ}\text{C}$  until analysis.

### 2.3. Analysis of blood lipids, serum glucose and CCK

Blood was drawn from the intra-orbital bullar plexus under isoflurane anesthesia and collected in ice-cold tubes. Serum was stored at  $-20^{\circ}\text{C}$  until analysis. Serum free fatty acids were measured by a NEFA C kit (Wako Chemicals GmbH, Neuss, Germany). Serum glucose was analyzed by Infinity™ Glucose Oxidase Liquid Stable Reagent (Thermo Electron, Melbourne Australia). Plasma CCK concentrations were measured by

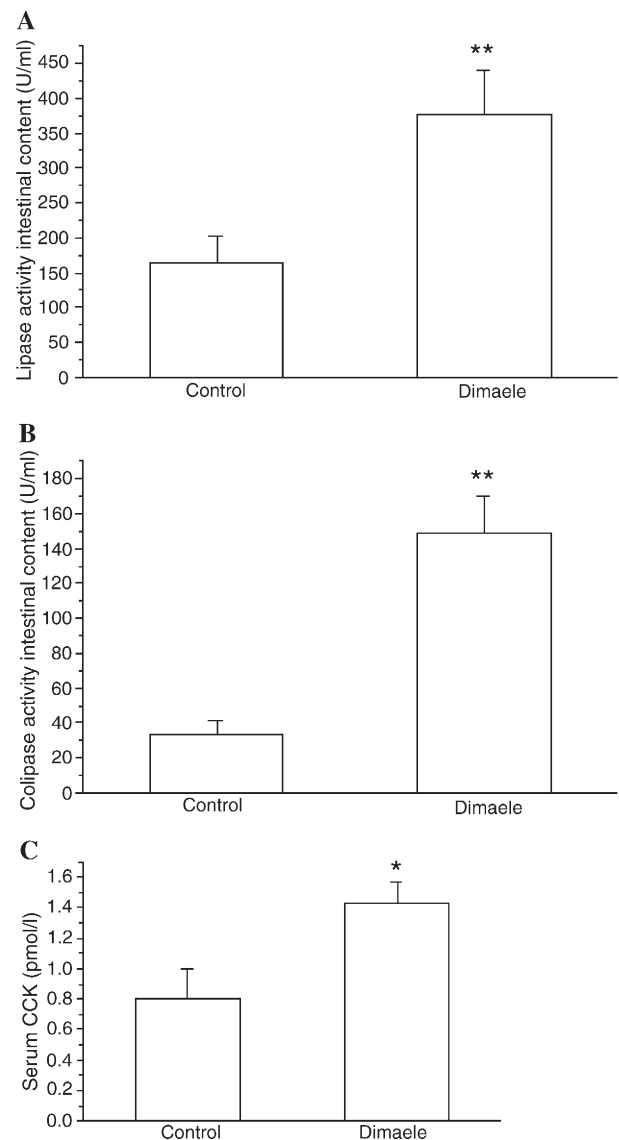


Fig. 4. Rats were given a single dose of dimaele (445  $\mu$ mol) plus 1 ml Intralipid (200 mg/ml) through oral gavage. The control rats received 1 ml Intralipid. Twelve hours after infusion, intestinal content was collected and lipase (A) and colipase activity (B) was analyzed by pH Stat. Fasting blood samples were collected and plasma CCK levels (C) analyzed by radioimmunoassay (RIA). Dimaele increased significantly lipase and colipase activity as well as plasma CCK levels.

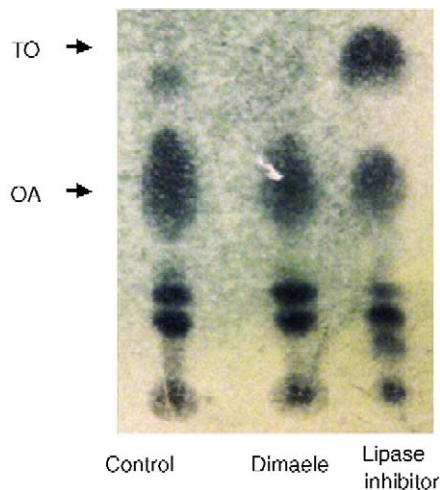


Fig. 5. Fecal fat analysis after feeding a high-fat diet for 6 days in control rat (1% methylcellulose), in rats receiving daily administration of dimaele (445  $\mu\text{mol}$ ) or a lipase inhibitor (Orlistat) (40 mg) [15]. Feces were collected and fecal fat was separated into lipid classes by thin layer chromatography (TLC). The position of the lipid standards are given on the left of the panel. TO, triolein, OA, oleic acid. Orlistat treatment results in steatorrhea, denoted by the presence of unhydrolysed triolein. The figure is a representative TLC-plate from three individual experiments.

radioimmunoassay using an antibody that does not display any cross-reactivity with gastrin [16].

#### 2.4. Analysis of pancreatic lipase and colipase in intestinal content

Lipase and colipase activity from intestinal content was determined using pH-stat titration (Mettler Components DK 10, DK 11, DV11) and tributyrin dispersed in bile salt as substrate [17]. Pure pancreatic lipase and colipase used for the inhibition experiments was purified according to previous techniques [18]. When Intralipid was used as substrate, the temperature of the reaction was 37  $^{\circ}\text{C}$ , otherwise room temperature was used.

#### 2.5. Fecal fat analysis

Feces were collected from all animals after 6 days of feeding the high-fat diet (long-term study). Lipids were extracted in chloroform/methanol (2:1) and the filtrates dried and weighed as previously described [19]. Pooled samples from all groups were analysed on a Silica G TLC plate, using triolein and oleic acid as standards. The plate was developed as previously described [19].

#### 2.6. Statistics

The StatView software (SAS Institute Inc., USA) was used for statistical analysis. The data are presented as mean  $\pm$  S.E.M. The results are analyzed using unpaired Student's *t*-test for comparisons of the dose dependence effect of dimaele. Two-way analysis of variance (ANOVA) was used for analysis of main effect of dimaele and interactions followed by post hoc

test (Bonferroni/Dunn) for comparison of individual differences. Statistical significance was set as  $*p < 0.05$ ,  $**p < 0.01$ ,  $***p < 0.005$ .

### 3. Results

#### 3.1. Inhibition of lipase/colipase activity in vitro

Based on protein/lipid interaction studies with purified lipase/colipase [9,20] a positively charged lipid compound was identified (dimaele) that inhibited the lipase–colipase mediated hydrolysis of triglycerides *in vitro* (Fig. 1). There was no effect of dimaele on lipase activity alone, when measured in the absence of bile salt (data not shown), but lipase–colipase activity was dramatically decreased in the presence of bile salt (Fig. 1A and B). Thus, the compound could be classified as a colipase inhibitor. The inhibition occurred both using a long-chain triglyceride, (Intralipid) emulsified with phospholipids and bile salt (Fig. 1A), and a short-chain triglyceride, (tributyrin) (Fig. 1B) dispersed in bile salt.

#### 3.2. Dose–response effect of dimaele on high-fat food intake in rat

The effect of dimaele was studied in rats fed a high-fat diet, containing 42% fat by energy and a low-fat diet, containing 15% fat by energy. Dimaele was given daily through oral gavage for 6 days. As seen in Fig. 2A, there was a significant and dose-dependent decrease in daily food intake after dimaele administration ( $p < 0.05$  and  $p < 0.01$ ). In rats fed low-fat diet there was a significant reduction in daily food but it was smaller and was not dose-dependent (Fig. 2B). In rats fed high-fat diet 445  $\mu\text{mol}$  of dimaele resulted in a reduction of food intake by approximately 50% (Fig. 2A), whereas with low-fat diet the same dose of dimaele gave a reduction of food intake by 15% (Fig. 2B). The time course of daily feeding is shown in Fig. 2C. Dimaele when given at a dose of 445  $\mu\text{mol}$  suppressed food intake at day 1 after infusion of dimaele, the suppression being maintained during the 6-day study period (Fig. 2C) (two-way ANOVA:  $F_{(2,607)} = 50.26$ ,  $p < 0.0001$  for the main effect of dimaele).

#### 3.3. Long-term effect of dimaele on blood glucose and lipids

After 6 days of infusion with dimaele, blood samples were collected from the rats that had fasted for 14 h. Serum glucose levels were reduced in rats receiving dimaele ( $p < 0.005$ ) compared to the control rats (Fig. 3A). Serum free fatty acids levels were reduced in the dimaele-treated animals compared to control animals ( $p < 0.01$ , Fig. 3B).

#### 3.4. Short-term effect of dimaele on lipase/colipase levels in intestinal content and on serum CCK-levels

As seen in Fig. 4 administration of dimaele stimulated the secretion of both lipase (Fig. 4A) and colipase (Fig. 4B) compared to control. Serum CCK levels were significantly elevated

following administration of dimaele compared to control ( $p < 0.05$ ; Fig. 4C).

### 3.5. Fecal fat analysis of animals treated with dimaele and a lipase inhibitor

The effects of dimaele during high-fat feeding for 6 days was further analysed through measurements of fecal fat composition. Fig. 5 shows a representative thin layer chromatography from three individual experiments. In contrast to the control, there was no sign of steatorrhea (indicated by the absence of the TO-spot) in the rats treated with dimaele. As a positive control rats treated with a lipase inhibitor (Orlistat 40 mg) demonstrated a steatorrhea.

## 4. Discussion

In this report we provide evidence for the concept that a compound that retards fat digestion also evokes satiety for fat. The compound used is a positively charged non-hydrolysable lipid, named dimaele, (*dimethylaminoethyl*dodecylether) that *in vitro* was found to inhibit the lipase–colipase mediated hydrolysis of triglycerides. This compound, that has the characteristics of a colipase inhibitor rather than a lipase inhibitor, furthermore failed to produce any steatorrhea (Fig. 5), as was demonstrated for the lipase inhibitor Orlistat, in agreement with previous findings [12]. Instead the secretion of pancreatic lipase and pancreatic colipase was greatly stimulated, to compensate for the impaired lipolysis (Fig. 4). This is the first time such a stimulatory compensation is described, acting to restore intraintestinal fat digestion and fat absorption.

The elevated secretion of pancreatic enzymes during treatment with dimaele probably occurred through the stimulation of CCK (Fig. 4C). At the same time the elevated levels of CCK might explain the satiety observed in these animals [21,22]. Another potential candidate molecule for satiety during fat intake is enterostatin, which is produced from pancreatic procolipase in equimolar amounts to colipase in the intestine [23]. The elevation of intestinal colipase (Fig. 4B) by dimaele suggests that also enterostatin is elevated, which in turn promotes satiety for fat [4].

Several control experiments were performed to determine if the reduced food intake by dimaele was due to malaise. With low-fat diet, containing 15% by energy as fat, there was a much weaker suppression of food intake with dimaele compared to the effect of dimaele with high-fat diet, containing 42% fat by energy (Fig. 2B), suggesting that the appetite suppression was tightly linked to the fat content of the diet and the impaired digestion of dietary fat (Fig. 2). There was no inflammatory response observed in the stomach and in the liver (data not shown). Further investigations will be performed to gain direct evidence that dimaele-induced suppression of food intake is not due to anorexia action.

Gastrointestinal mechanisms to express satiety consist of humoral as well as neuronal signals [8]. Whereas gastric satiety is induced by a mechanical stimulus, such as increased volume, small intestinal satiety is nutrient-specific and provides a me-

chanism for sustained satiety. Small intestinal satiety is largely mediated through CCK mechanisms, as demonstrated for fat primarily [24,25]. There is also an indication that fatty acids and monoacylglycerol are more satiating than unhydrolysed fat [26]. Thus a compound that reduces the rate of fat digestion, without leaving unhydrolysed fat in the intestine, like dimaele, is more satiating than a compound that yields unhydrolysed fat in the intestine like Orlistat [27,28].

Taken together our data demonstrate the unique advantage of a specific colipase inhibitor (dimaele) over a general lipase inhibitor (Orlistat) in controlling appetite for fat. The compound provides a novel approach for the treatment of obesity and related metabolic disorders. The advantage of such a compound is also that it does not interfere with central mechanisms to regulate feeding, which are closely related to the reward system.

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