British Journal of Nutrition (2003), **90**, 207–214
DOI: 10.1079/BJN2003871

The effects of enterostatin intake on food intake and energy expenditure

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(Received 14 December 2001 – Revised 5 February 2003 – Accepted 20 February 2003)

Enterostatin (ENT) has been found to inhibit food intake and selectively inhibit fat intake in rats. Both peripheral and central mechanisms have been proposed. It also has been suggested that ENT may increase thermogenesis. The present study investigated the effects of oral ENT administration on food intake, energy expenditure and body weight in subjects with a preference for a high-fat diet. In a double-blind, placebo-controlled, randomized and crossover design, nine female and three male healthy subjects (age 34 (SD 11) years, BMI 24·5 (SD 2·5) kg/m^2) with a preference for a high-fat diet ingested ENT (3 × 15 mg/d) or placebo (PLA) while consuming a high-fat diet *ad libitum* for 4 d. Eight subjects ended each intervention with a 36 h stay in the respiration chamber, continuing the diet and treatment. Body-weight loss was significant (ENT 0·8 (SE 0·3) kg, $P<0·05$; PLA 1·3 (SE 0·3) kg, $P<0·001$), but not different between treatments. There was no difference between treatments in total energy intake (ENT 37·1 (SE 2·6), PLA 35·9 (SE 3·2) MJ), macronutrient composition, hunger, satiety and hedonic scores during the 4 d high-fat diet. Energy expenditure (24 h) (ENT 9·6 (SE 0·4), PLA 9·5 (SE 0·4) MJ), sleeping and resting metabolic rate, diet-induced thermogenesis, activity-induced energy expenditure and 24 h RQ (ENT 0·77 (SE 0·01), PLA 0·77 (SE 0·01)) were similar for both treatments. We conclude that oral ENT administration did not affect food intake, energy expenditure or body weight in subjects with a preference for a high-fat diet experiencing a negative energy and fat balance.

Energy intake: Fat intake: Energy expenditure: Body weight: Fat preference

A high-energy intake (EI), often caused by a high-fat intake together with the possible inability to couple fat oxidation to fat intake for energy balance, has been found to be related to obesity in human subjects (Swinburn & Ravusssin, 1993; Schutz, 1995). Selective inhibition of fat intake can be a useful approach to reduce total EI.

Recently, a growing list of peptides has been shown to inhibit EI (Bray, 2000). Enterostatin (ENT), a pentapeptide produced by trypsin cleavage of pancreatic procolipase in the intestine, is one of those peptides. ENT administration has been found to reduce EI and to selectively inhibit fat intake in rats in a dose-dependent manner (Erlanson-Albertsson *et al.* 1991; Okada *et al.* 1991; Sörhede *et al.* 1993; Lin *et al.* 1997). However, the exact mechanism by which ENT causes its effects is unclear. Both peripheral and central sites of action have been proposed. The peripheral response is mediated via the afferent vagus (Tian *et al.* 1994). The central response is thought to be mediated through both opioid and serotonergic pathways (Lin & York, 1994; Ookuma *et al.* 1997).

Chronic administration of ENT has been found to reduce body weight and body fat in rats (Okada *et al.* 1993; Lin *et al.* 1997). Besides effects on food intake, metabolic effects, such as reduction of insulin secretion (Mei *et al.* 1993; Okada *et al.* 1993; Mei & Erlanson-Albertsson, 1996), stimulation of corticosterone secretion (Okada *et al.* 1993) and stimulation of brown adipose tissue (Nagase *et al.* 1997) have also been suggested to contribute to body-weight and body-fat loss.

In human subjects, it is suggested that ENT secretion may be reduced in obesity, in both a fasting and a post-ingestive state (Bowyer *et al.* 1993; Prasad *et al.* 1999). However, only a few studies have investigated the effects of ENT administration on food intake and appetite in human subjects (Rössner *et al.* 1995; Smeets *et al.* 1999). It has been suggested that only human subjects who express a particular preference for fat will be responders to ENT (Rössner *et al.* 1995). In rats it has been observed that chronic ingestion of fat is required for the response to ENT (Lin & York, 1998). However, the mechanism through which chronic ingestion of fat increases the susceptibility to ENT is not known. A range of endocrine, metabolic and neurochemical changes due to fat ingestion may be involved.

The aim of the present study was to investigate the effects of oral ENT administration on EI, energy expenditure (EE) and body weight in human subjects. This was studied in subjects with a preference for a high-fat diet. We hypothesized that ENT would reduce the rewarding value of fat consumption. We therefore offered the subjects

Abbreviations: EE, energy expenditure; EI, energy intake; ENT, enterostatin; PLA, placebo.

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a high-fat diet for 4 d, expecting that the reduction in rewarding value for fat would decrease EI beyond a decrease through sensory-specific satiety. This would result in a relatively larger decrease in total EI, ultimately leading to a greater body-weight reduction.

Methods

Subjects

The subjects were recruited by advertisements on boards at Maastricht University or in local newspapers, in which we asked for men and women who enjoy eating. Forty-five subjects were invited for a pre-test in order to make a distinction between subjects with a preference for a high-fat diet (fat cravers) and subjects with no preference for a high-fat diet (non-fat cravers). This was done using a macronutrient-specific food-frequency questionnaire and a macronutrient-specific food-choice test. Development of the macronutrient-specific food-frequency questionnaire and the macronutrient-specific food-choice test and determination of the selection criteria to indicate whether subjects were fat cravers or non-fat cravers were carried out separately in a previous test. Subjects were selected on being healthy (no diabetes, no gastrointestinal or cardiovascular diseases and no medical treatment) and on having a BMI 22–30 kg/m^2. Nine female and three male subjects were selected for participation in the study. Baseline characteristics of the subjects are presented in Table 1. The nature and risks of the experimental procedure were explained to the subjects, and all subjects gave their written informed consent. The study was approved by the Ethical Committee of Maastricht University.

Experimental design

The experiment had a double-blind, placebo-controlled, randomized, crossover design. The intervention consisted of two periods of a 4 d high-fat diet with *ad libitum* food intake separated by a washout period of at least 2 weeks (4 d high-fat diet experiment). Since it has been suggested that EI is affected by the phase of the menstrual cycle, we aimed to measure all women in the same phase of their cycle. We chose to offer the subjects a high-fat diet because it has been previously shown in rats that ENT reduces food intake only when the fat content of a diet is high (Erlanson-Albertsson *et al.* 1991; Okada *et al.* 1992; Lin *et al.* 1997).

From the evening of the fourth day until the morning of the sixth day, a sub-sample of six female and two male subjects stayed in a respiration chamber for 36 h. Measurements of EE and substrate oxidation were performed on the fifth day (respiration chamber experiment). The subjects ingested ENT or placebo (PLA) during 4 or 5 d of intervention respectively.

Interventions

Food intake during the 4 d high-fat diet period. The high-fat diet that was provided was composed of 60 % energy as fat, 25 % as carbohydrate and 15 % as protein. The subjects came to the University Eating Laboratory for breakfast, lunch and dinner, always at the same time of the day. Breakfast consisted of 'bites' of 1 g thin crispbread with 5 g meat (cervelat sausage) or cheese (gouda), having similar macronutrient composition (eighteen 'bites': energy 1·74 MJ, protein 21 g, carbohydrate 14 g, fat 31 g). Lunch consisted of 'bites' of 1 g thin crispbread with 5 g meat (cervelat sausage or liver sausage) or cheese (Brie or Port Salut), all having similar macronutrient composition (eighteen 'bites': energy 1·62 MJ, protein 19 g, carbohydrate 14 g, fat 28 g). Dinner consisted of different small party snacks (ball-shaped meat croquettes, meat balls and cheese croquettes) (eighteen snacks: energy 7·35 MJ, protein 68 g, carbohydrate 79 g, fat 132 g),

Table 1. Characteristics of subjects at baseline*

	Mean	SD	Range
Age (years)	34	11	21–49
Height (m)	1·73	0·12	1·54–1·98
Weight (kg)	73·10	11·48	54·06–96·02
BMI (kg/m^2)	24·5	2·5	22·1–29·8
Waist circumference (m)	0·82	0·11	0·7–1·02
Hip circumference (m)	1·01	0·05	0·93–1·06
Waist:hip ratio	0·81	0·08	0·73–0·98
Body fat (%)	28·3	6·1	16·2–40·9
Attitude towards eating			
Three-factor eating questionnaire†			
F1 (cognitive restraint)‡	5	5	1–14
F2 (disinhibition)‡	4	4	1–13
F3 (hunger)‡	5	4	0–11
Herman–Polivy restraint score‡§	12	6	5–23
High-fat diet preference			
Macronutrient-specific food-frequency questionnaire score‖	0·49	0·01	0·48–0·52
Macronutrient-specific food-choice test score‖	85	14	70–100

* *n* 12 (three men, nine women).
† For details, see Stunkard & Messick (1985) and Westerterp-Plantenga *et al.* (1999).
‡ For Herman–Polivy restraint (normal <15) and F1–F3 scores (normal <9), a higher value indicates more restraint, disinhibition, hunger.
§ For details, see Herman & Polivy (1980).
‖ For details, see p. 209.

served with a cucumber (100 g) and tomato (100 g) salad. Food intake during breakfast, lunch and dinner was *ad libitum* with the exception of the cucumber and tomato salad. If the amount of food served was not enough, extra 'bites' were offered to the subjects. Snacks consisting of crisps and cashew nuts were consumed *ad libitum* in the morning, in the afternoon between meals and in the evening. The subjects had to consume an apple in the afternoon. The subjects were not allowed to eat and drink anything else in addition to the prescribed food items, with the exception of water, coffee and tea (without sugar and milk).

Food supplements. During the high-fat diet, three times per d, the subjects ingested a capsule containing: ENT treatment, 15 mg ENT (alanine–proline–glycine–proline–arginine; PolyPeptide Laboratories, Wolfenbüffel, Germany) and 485 mg lactose; PLA treatment, 500 mg lactose. This resulted in a daily intake of 45 mg or 0 mg ENT respectively. The capsules were ingested immediately before breakfast, lunch and dinner along with a glass of water. The timing of capsule ingestion immediately before the meals was chosen because it has been previously shown that hunger suppression is likely to appear soon after ENT ingestion (Smeets *et al.* 1999).

Measurements

Macronutrient preference test. Prior to the actual experiment, the macronutrient-specific food-frequency questionnaire and macronutrient-specific food-choice test were developed. Fifty-two subjects (forty-one females and eleven males) participated in two tests concerning a macronutrient-specific food-frequency questionnaire and a macronutrient-specific food-choice test. Their characteristics were: age 28 (SD 10) years, BMI 22·3 (SD 2·5) kg/m^2. They were dietary unrestrained, as indicated by the three-factor eating questionnaire (Stunkard & Messick, 1985; Westerterp-Plantenga *et al.* 1999) and the Herman-Polivy questionnaire (Herman & Polivy, 1980).

Macronutrient-specific food-frequency questionnaire. The subjects received a questionnaire that we have designed with questions about the frequency of eating high-fat and high-carbohydrate food items. The number of food items given in the questionnaire was the same (*n* 22) for high-fat and high-carbohydrate items. The answers to the questions were given according to a one- to four-point scale (1, never; 2, at least once per year; 3, at least once per month; 4, at least once per week). The mean frequency for eating high-fat or high-carbohydrate food items was calculated. The mean frequency for eating high-fat food items:mean frequency for eating (high-carbohydrate + high-fat food items) ratio was calculated. The ratio was 0·46 (SD 0·03) (range 0·38–0·51). The cut-off point for a preference for a high-fat diet was calculated using frequency distribution and was based on the highest tertile. The subjects in the highest tertile had a score $\geq$0·48 on the macronutrient-specific food-frequency questionnaire.

Macronutrient-specific food-choice test. The subjects came to the laboratory at lunchtime, at least 2 h after the last meal. They received a choice of high-carbohydrate and high-fat food items. The high-fat food items were halves of croissants with cheese or salami, or sausage rolls. The high-carbohydrate items were bread rolls with jam or fresh cheese, or currant buns. Food intake was *ad libitum* and the subjects were instructed to eat until they felt comfortable. They were allowed to drink tap water. The percentage of high-fat food items eaten from total food items was calculated. The subjects ate a mean value of 62 (SD 30) (range 0–100) % high-fat food items. The cut-off point for a preference for a high-fat diet was calculated using frequency distribution and was based on the highest tertile. The subjects in the highest tertile had a score $\geq$80 % on the macronutrient-specific food-choice test.

Determination of a preference for a high-fat diet (pre-test). The subjects received the macronutrient-specific food-frequency questionnaire. The mean frequency for eating high-fat or high-carbohydrate food items was calculated. If the values of the mean frequency for eating high-fat food items:mean frequency for eating high-carbohydrate + high-fat food items was $\geq$0·48, the subjects were considered to be fat likers.

The subjects underwent the macronutrient-specific food-choice test. The percentage of high-fat food items eaten from total food items was calculated. Subjects who ate $\geq$ 80 % high-fat food items were considered fat likers.

Subjects who were found to be fat likers in both the macronutrient-specific food-frequency questionnaire and the macronutrient-specific food-choice test were considered fat cravers and were included in the main experiment.

Screening

Body weight and BMI. Body weight was measured on a digital balance (model 707, weighing accuracy of 0·1 kg; Seca, Hamburg, Germany) with subjects in underwear, in the fasted state and after voiding their bladder. Height was measured to the nearest 1 mm using a wall-mounted stadiometer (model 220; Seca). The BMI was calculated by body weight/height^2 (kg/m^2).

Fat distribution. The distribution of fat was investigated by measuring the waist and hip circumferences and calculation of the waist:hip ratio. The waist circumference was measured at the site of the smallest circumference between the rib cage and the ileac crest, with the subjects in standing position. The hip circumference was measured at the site of the largest circumference between the waist and the thighs. The waist:hip ratio was calculated by dividing the waist circumference by the hip circumference.

Body composition. Whole-body composition was determined by under-water weighing with simultaneous assessment of residual lung volume by the He-dilution technique using a spirometer (Volugraph 2000; Bunnik, Mijnhardt, The Netherlands). Measurements were performed in triplicate and the average was used to calculate body density.

Attitude towards eating. Attitude towards eating was determined using a validated Dutch translation of the three-factor eating questionnaire (Stunkard & Messick, 1985; Westerterp-Plantenga *et al.* 1999). Cognitive restrained and unrestrained eating behaviour (factor 1),

emotional eating and disinhibition (factor 2) and the subjective feeling of hunger (factor 3) were scored. Body-weight concern and chronic dieting behaviour were investigated with the Herman–Polivy questionnaire (Herman & Polivy, 1980).

4-d High-fat diet experiment

Energy intake. EI (24 h) and macronutrient composition were determined using the Dutch food composition tables (Stichting Nederlands Voedingsstoffenbestand, 1996) and the accessory computer program (Becel Nutrition Program, NL04A 1992; Unilever, Rotterdam, The Netherlands).

Hunger and satiety. Hunger and satiety were scored every day on anchored 100 mm visual analogue scales. Questionnaires were completed at ten fixed time points: immediately before and after breakfast, in the morning between 10.00 and 11.00 hours, immediately before and after lunch, in the afternoon at 15.00 hours, immediately before and after dinner, in the evening between 21.00 and 22.00 hours, and before sleeping.

Hedonics. Pleasantness of the taste of the food was scored every day on a one- to ten-point scale after the first bite of each food item consumed during breakfast, lunch and dinner.

Tolerance. Adverse events were recorded every day using a questionnaire on the occurrence of gastrointestinal and other complaints and the severity of the outcome was specified on a five-point scale (0, not at all; 1, less; 2, sometimes; 3, relatively much; 4, extremely).

Mood. Variables of mood (Lorr & McNair, 1984) were scored every day on anchored 100 mm visual analogue scales.

Respiration chamber experiment

Energy intake. Foods offered during the stay in the respiration chamber were similar to that of the previous 4 d. To determine the appropriate level of EI for attaining energy balance, sleeping metabolic rate was measured during the first night and multiplied by an activity index of 1·65 (Schrauwen *et al.* 1997).

Protocol. In the respiration chamber, subjects followed a protocol consisting of fixed times for breakfast (08.30 hours), lunch (12.00 hours), dinner (18.00 hours), and snacks (10.00, 15.00, 20.30 hours), sedentary activities and bench-stepping exercise. The bench-stepping exercise was performed three times per d (10.30, 14.30, 20.30 hours) for 30 min each time, consisting of intervals of 5 min exercise alternated with 5 min rest, at a rate of one step per s with a bench height of 0·25 m. Apart from the exercise protocols, subjects were not restricted in their activities, except that sleeping and strenuous physical activity were not allowed. Subjects were expected to sleep from 23.00 to 08.00 hours. Subjects further completed questionnaires on hunger and satiety, hedonics, tolerance, and mood, which were described earlier.

Indirect calorimetry. O_2 consumption and CO_2 production were measured in a respiration chamber (Schoffelen *et al.* 1997). The respiration chamber was a 14 m^3 room, furnished with a bed, chair, computer, television, radio–cassette player, telephone, intercom, sink and a toilet. The room was ventilated with fresh air at a rate of 70–80 l/min. The ventilation rate was measured with a dry-gas meter (Schlumberger, type 4; Schlumberger, Dordrecht, The Netherlands). The concentrations of O_2 and CO_2 were measured using a paramagnetic O_2 analyser (Magnos 6G; Hartmann & Braun, Frankfurt, Germany; type 4100; Servomex Controls Ltd, Crowbrough, Sussex, UK) and an infrared CO_2 analyser (Uras 3G; Hartmann and Braun). During each 15 min period six samples of outgoing air for each chamber and one sample of fresh air, zero gas and calibration gas were measured. The gas samples to be measured were selected by a computer that also stored and processed the data (Schoffelen *et al.* 1997).

Energy expenditure and substrate oxidation. EE (24 h) consisted of sleeping metabolic rate, diet-induced thermogenesis and activity-induced EE. EE and RQ (24 h) were calculated from 07.00 to 07.00 hours, from O_2 consumption and CO_2 production according to the formulas of Weir (1949).

Physical activity was monitored using a radar system working on the Doppler principle (Advisor DU 160; electronically adapted in own laboratory).

Sleeping metabolic rate was defined as the lowest mean EE measured over 3 h consecutively, between 00.00 and 07.00 hours. Sleeping metabolic rate was measured on both nights and the average of the two nights was used for further calculations. Diet-induced thermogenesis was calculated by plotting EE against radar output, both averaged over 30 min periods. The intercept of the regression line at the lowest radar output represented the EE in the inactive state (resting metabolic rate), consisting of sleeping metabolic rate and diet-induced thermogenesis (Westerterp *et al.* 1998). Diet-induced thermogenesis was determined by subtracting sleeping metabolic rate from resting metabolic rate. Activity-induced EE was determined by subtracting resting metabolic rate from 24 h EE. Physical activity level was calculated by dividing 24 h EE by sleeping metabolic rate.

Carbohydrate, fat, and protein oxidation were calculated using O_2 consumption and CO_2 production and urinary N excretion, using the formulas of Brouwer (1957):

$$\text{protein oxidation (g/d)} = 6{\cdot}25 \times \text{N},$$

$$\text{fat oxidation (g/d)} = 1{\cdot}718 \times V_{O_2} - 1{\cdot}718 \times V_{CO_2} - 0{\cdot}315 \times \text{P}$$

and

$$\text{carbohydrate oxidation (g/d)} = 4{\cdot}17 \times V_{CO_2} - 2{\cdot}965 \times V_{O_2} - 0{\cdot}390 \times \text{P},$$

where N is total N excreted in urine (g/d), V_{O_2} is oxygen consumption (litres/d), V_{CO_2} is carbon dioxide production (litres/d) and P is protein oxidation (g/d).

A 24 h urine collection was made from the second voiding on the day of measurement in the respiration chamber until the first voiding of the following day. Samples were collected in containers with 10 ml H_2SO_4 to prevent N loss through evaporation. Volume and N concentration

were measured, the latter using a N analyser (Elemental Analyzer, CHN-O-Rapid; Heraeus, Hanav, Germany).

Data analysis

Data are presented as mean values with their standard errors. Differences between the two treatments were determined using paired *t* test. Pearson correlation coefficients (*r*) were calculated to determine the relationship between selected variables. The level of significance was set at $P<0{\cdot}05$. All statistics were executed with STATVIEW™ SE+GRAPHICS (1988; Abacus Concepts, Inc., Berkeley, CA, USA).

Results

There was a significant body-weight loss during the 4 d high-fat diet (ENT 0·8 (SE 0·3) kg, $P<0{\cdot}05$; PLA 1·3 (SE 0·3) kg, $P<0{\cdot}001$), with no significant difference between treatments.

EI (24 h) during the 4 d high-fat diet is given in Table 2. There was no difference in total EI during the 4 d high-fat diet (ENT 37·1 (SE 2·6), PLA 35·9 (SE 3·2) MJ) or during each day separately. The subjects were in negative energy balance, as shown by body-weight loss. There was no significant correlation between EI, calculated as % predicted EE (Harris & Benedict, 1919), and factor 1 of the three-factor eating questionnaire. Macronutrient composition was 64 % energy as fat, 20 % as carbohydrate and 16 % as protein on each day. In addition, there were no differences in EI and macronutrient composition from the different meals (breakfast, lunch, dinner and snacks).

Mean hunger and satiety scores on each day or over the 4 d high-fat diet (ENT 34 (SE 3), PLA 34 (SE 4) mm and ENT 58 (SE 3), PLA 58 (SE 4) mm respectively) were not different between treatments.

Hedonics, measured as mean score on each day and over the 4 d high-fat diet (ENT 7·4 (SE 0·2), PLA 7·4 (SE 0·2)), were not different between treatments. Hedonic scores did not change over the 4 d (ENT 0·1 (SE 0·1), PLA −0·2 (SE 0·2)).

Occurrence of gastrointestinal and other complaints was low and not different between treatments, with the exception of 'headache' and 'constipation' that tended to be lower with ENT compared with PLA, as shown by the daily mean score over the 4 d high-fat diet (headache: ENT 0·3 (SE 0·1), PLA 0·8 (SE 0·2), $P<0{\cdot}1$; constipation: ENT 0·3 (SE 0·2), PLA 0·5 (SE 0·3), $P<0{\cdot}1$).

The subjects felt more relaxed (ENT 83 (SE 4), PLA 75 (SE 5) mm, $P<0{\cdot}05$) and less gloomy (ENT 7 (SE 3), PLA 16 (SE 4) mm, $P<0{\cdot}05$) with ENT compared with PLA, as shown by the mean total scores measured during the 4 d high-fat diet. This was caused by a higher baseline score for 'relaxed' (ENT 81 (SE 6), PLA 68 (SE 7) mm, $P<0{\cdot}05$) and a lower score for 'gloomy' (ENT 5 (SE 2), PLA 20 (SE 8) mm, $P<0{\cdot}05$) with ENT compared with PLA on day 1. No difference between treatments was found for the other mood variables.

EI (24 h) on day 5 during the stay in the respiration chamber was similar for both treatments (ENT 10·3 (SE 0·5), PLA 10·2 (SE 0·6) MJ). Macronutrient composition was 66 % energy as fat, 18 % as carbohydrate and 16 % as protein; the food quotient was 0·77. Fat intake was similar for both treatments (Table 3).

EI (24 h) on day 5 was higher compared with day 4 (ENT 7·6 (SE 0·7) MJ, $P<0{\cdot}001$; PLA 7·2 (SE 0·8) MJ, $P<0{\cdot}001$). Mean hunger score on day 5 (ENT 20 (SE 4), PLA 22 (SE 5) mm) was significantly lower compared with day 4 (ENT 30 (SE 5) mm, $P<0{\cdot}05$; PLA 32 (SE 6) mm, $P<0{\cdot}01$). Similarly, mean satiety score on day 5 (ENT 69 (SE 4) mm; PLA 69 (SE 6) mm) was significantly higher compared with day 4 (ENT 60 (SE 6) mm, $P<0{\cdot}1$; PLA 61 (SE 70) mm, $P<0{\cdot}01$). Mean hedonic score tended to be lower on day 5 (ENT 6·9 (SE 0·4), PLA 6·7 (SE 0·4)) compared with day 4 (ENT 7·3 (SE 0·2), $P<0{\cdot}1$; PLA 7·1 (SE 0·4), $P<0{\cdot}1$).

EE (24 h) was similar for both treatments (ENT 9·6 (SE 0·4), PLA 9·5 (SE 0·4) MJ), indicating that the subjects were in positive energy balance of 0·7 (SE 0·3) MJ for both treatments during the stay in the respiration chamber. There were no differences in sleeping metabolic rate, diet-induced thermogenesis, resting metabolic rate, activity-induced EE (Fig. 1) and physical activity level between treatments. There were also no differences in RQ (ENT 0·77 (SE 0·01), PLA 0·77 (SE 0·01)) and non-protein RQ (ENT 0·76 (SE 0·01), PLA 0·76 (SE 0·01)). Fat oxidation and fat balance were similar for both treatments (Table 3). Sleeping metabolic rate, resting metabolic rate and 24 h EE were significantly related to fat-free mass in both treatments (Table 4).

Table 2. Energy intake during the high-fat diet (MJ/24 h) in subjects given enterostatin or placebo*

(Mean values with their standard errors for eight subjects)

Treatment...	PLA		ENT†		
	Mean	SE	Mean	SE	Statistical significance of effect: *P*‡
Day 1	8·7	0·8	9·0	0·8	NS
Day 2	9·2	0·8	9·6	0·5	NS
Day 3	8·9	0·9	9·4	0·7	NS
Day 4	9·1	1·0	9·0	0·8	NS

PLA, placebo; ENT, enterostatin.

* For details of subjects, treatments and procedures, see Table 1 and p. 208.

† 45 mg/d; PolyPeptide Laboratories, Wolfenbüffel, Germany.

‡ Paired two-tailed *t* test.

Table 3. Fat intake, fat oxidation and fat balance (g/d) during the stay in the respiration chamber in subjects given enterostatin or placebo*
(Mean values with their standard errors for eight subjects)

Treatment...	PLA		ENT†		
	Mean	SE	Mean	SE	Statistical significance of effect: *P*‡
Fat intake	178·8	10·2	180·9	9·7	NS
Fat oxidation	108·0	7·2	111·4	4·0	NS
Fat balance	70·8	12·8	69·5	9·5	NS

PLA, placebo; ENT, enterostatin.
* For details of subjects, treatments and procedures, see Table 1 and p. 208.
† 45 mg/d; PolyPeptide Laboratories, Wolfenbüffel, Germany.
‡ Paired two-tailed *t* test.

Discussion

In the present study, we did not find an effect of oral ENT administration on food intake, appetite or body weight. This is in contrast to animal studies, where promising results on the effects of ENT on food and fat intake have been shown during normal feeding and in experimental paradigms that involve dietary choice (Erlanson-Albertsson *et al.* 1991; Okada *et al.* 1991; Sörhede *et al.* 1993; Lin *et al.* 1997). However, the exact mechanism by which ENT provokes its effects is still unclear. It has been suggested that both peripheral and central sites of action may be involved. The peripheral mechanism involves an afferent vagal signalling pathway to hypothalamic centres (Tian *et al.* 1994). The central response is thought to be mediated through pathways including both serotonergic and κ-opiodergic components (Lin & York, 1994; Ookuma *et al.* 1997). These mechanisms might have an important role in the selection and appreciation of macronutrients, in this case fat.

Until now, only a few studies have investigated the effects of ENT administration on food intake and appetite in human subjects (Rössner *et al.* 1995; Smeets *et al.* 1999). Rössner *et al.* (1995) did not observe any effect of intravenous ENT administration (4 and 16 mg) in healthy obese male subjects on subsequent food intake during a test meal. They speculated that the lack of an effect may be due to the inability of intravenous ENT to reach the site of action, the time between ENT administration and the subsequent meal (5 min), and the possibility that human responders would be those who have a particular preference for fat.

In this respect, Lin & York (1998) observed in rats that chronic ingestion of dietary fat is a prerequisite for the inhibiting action of ENT on feeding. They suggested that chronic ingestion of fat initiates metabolic, endocrine or neurochemical changes that are required for the response to ENT. For this reason, subjects with a preference for a high-fat diet were selected in the present study. Furthermore, it has been previously shown in rats that ENT reduces food intake of single- and two-choice diets when the fat content is high, but not when the fat content is low (Erlanson-Albertsson *et al.* 1991; Okada *et al.* 1992; Lin *et al.* 1997). Therefore, a high-fat diet was used together with the treatment in the present study. Smeets *et al.* (1999) did not find any effect of oral ENT administration (4 and 16 mg) in healthy male subjects ranging from normal weight to obese on subsequent food intake during a test meal presented after 60 min. However, they observed that hunger was significantly reduced 30 min after oral

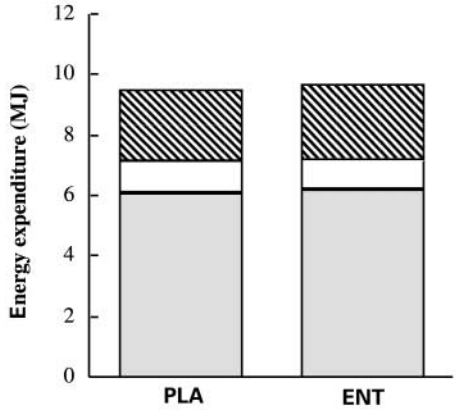

Fig. 1. Energy expenditure (24 h) and components of energy expenditure in subjects given enterostatin (ENT) (45 mg/d; PolyPeptide Laboratories, Wolfenbüffel, Germany) or placebo (PLA). ▨, Sleeping metabolic rate; □, diet-induced themogenesis; ▧, activity-induced energy expenditure. For details of subjects and procedures, see Table 1 and p. 208. Values are means for eight subjects. Statistical significance between treatments was determined by paired two-tailed *t* test.

Table 4. Relationship between energy expenditure and fat-free mass in subjects given enterostatin or placebo* (Pearson's correlation coefficients for eight subjects)

Treatment...	PLA		ENT†	
	r	*P*	*r*	*P*
SMR *v.* FFM	0·90	<0·05	0·93	<0·01
RMR *v.* FFM	0·88	<0·05	0·86	<0·05
24 h EE *v.* FFM	0·93	<0·01	0·87	<0·05

PLA, placebo; ENT, enterostatin; SMR, sleeping metabolic rate; FFM, fat-free mass; RMR, resting metabolic rate; EE, energy expenditure.
* For details of subjects, treatments and procedures, see Table 1 and p. 208.
† 45 mg/d; PolyPeptide Laboratories, Wolfenbüffel, Germany.

ingestion with both doses of ENT but not with PLA. After 60 min, hunger remained significantly reduced only with the 16 mg dose, suggesting that hunger suppression is likely to appear early after ENT ingestion. Furthermore, it has been shown in rats that with the exception of intravenous administration, the action of ENT is rapid (< 30 min) after administration by other routes, i.e. intraduodenally, intraperitoneally and intracerebroventricularly (Erlanson-Albertsson & York, 1997). This explains the rationale for the timing of capsule ingestion immediately before the meals in the present study.

Results suggest that obese individuals are probably more likely to be sensitive to ENT administration compared with normal-weight subjects. Individuals who do not show increased ENT secretion following a meal are the most likely to show reduced food intake after ENT administration. In this respect, it would be useful to investigate ENT secretion in different individuals in order to identify ENT-sensitive subjects. Because an assay sensitive enough to determine ENT concentrations in serum was lacking until recently, individual ENT secretion has been poorly investigated (Erlanson-Albertsson & York, 1997). Procolipase activity, measured in the duodenal content after cholecystokinin administration, is reduced in obese compared with normal-weight subjects. ENT immunoreactivity can be detected in serum of normal weight, but not of obese individuals. ENT concentration in urine is significantly less in obese compared with normal-weight subjects (Erlanson-Albertsson & York, 1997). In the present study, the subjects had a BMI between 22 and 30 kg/m^2. Because these subjects were normal weight to overweight, it might be possible that they were not sensitive enough for a response to ENT. Due to the rigid selection criteria, we were not able to include a larger number of subjects in order to look at a relationship between BMI or body fat and the response to ENT. Furthermore, the negative energy balance may have contributed to the lack of a response to ENT. Although the diet was high in fat, it might be possible that the absolute amount of fat ingested was too small to induce an effect of ENT.

In the present study, we did not find a metabolic effect as a result of ENT treatment. EE (24 h) and the components of EE measured during a 36 h stay in a respiration chamber at the end of a 4 d high-fat diet were not affected by ENT administration. This is in contrast to animal studies, where it has been found that several metabolic effects may be involved in the reduction of body weight and body fat. ENT has been found to decrease insulin secretion (Mei *et al.* 1993; Okada *et al.* 1993; Mei & Erlanson-Albertsson, 1996) and to stimulate corticosterone secretion (Okada *et al.* 1993). This will promote the catabolic effects of glucocorticoids and stimulate adipose tissue lipolysis. In the present study, we did not measure insulin and corticosterone concentrations in blood, so we are not able to draw a conclusion in this regard. In addition to the endocrine effects, ENT has been found to activate the sympathetic drive to brown adipose tissue, which would be expected to increase thermogenesis (Nagase *et al.* 1997). Because brown adipose tissue is limited in adult human subects, this might explain why we did not observe any effect of ENT on thermogenesis in our subjects.

During the *ad libitum* 4 d high-fat diet, the subjects were in a negative energy balance. EI was 78 % predicted EE. The discrepancy between actual and expected EI was not due to restrained eating behaviour, as there was no relationship between EI as % predicted EE and factor 1 of the three-factor eating questionnaire. The negative EI during the intervention period was probably due to a specific and imposed food–fat intake pattern that was not familiar to the subjects. In contrast, the subjects were in positive energy balance on day 5 during the stay in the respiration chamber, when the EI was fixed. EI was 107 % EE measured in the respiration chamber. One explanation might be that the subjects were relatively inactive in the respiration chamber and that therefore the activity index used to calculate EI from sleeping metabolic rate was too great.

Some variables of mood were more positive in the ENT compared with the PLA treatment. This may suggest that ENT might have an effect on the opioid system and that this would influence mood. However, the differences in mood variables between the treatments were already present at baseline on day 1, indicating that the differences were not due to the treatment, but already existed before the intervention started.

During the 4 d high-fat diet, we did not observe a decrease in hedonics. This indicates that in this experiment we did not detect a sensory-specific satiety effect of fat. However hedonic scores were lower on day 5 during the stay in the respiration chamber compared with the previous days in both treatments. This is likely to be caused by overconsumption on day 5, which resulted in increased satiety and decreased hunger scores and subsequently in lower hedonic scores.

It is known that oral administration of peptides and proteins is often limited because of their instability in the gastrointestinal environment. Therefore, it might be argued that ENT administered orally will lose its effects when passing through the stomach, as a result of deactivation by enzymes. In this respect, it would be appropriate to administer ENT in capsules that release their contents within the lumen of the duodenum. However, an effect of ENT on appetite was found also when administered orally (Smeets *et al.* 1999). Furthermore, ENT has been found not only to be formed in the intestine by the cleavage of secreted pancreatic procolipase, but also to be produced in the gastric mucosa and the mucosal epithelia of the small intestine (Erlanson-Albertsson & York, 1997).

It may be argued that the results may have been affected by the different phases of the menstrual cycle in which the measurements were completed. With regard to this, it has been shown that chocolate is a frequently craved food during the premenstrual phase (Tomelleri & Grunewald, 1987; Rozin *et al.* 1991) and that increases in fat intake may thus account for the premenstrual increases in EI (Tarasuk & Beaton, 1991). On the other hand, non-premenstrual syndrome sufferers showed no evidence of cyclic changes in intake of fat *v.* carbohydrate *v.* protein (Jas, 1996), and although these women reported that they often craved chocolate premenstrually, they did not eat more chocolate at this time (Jas, 1996).

In the present study, six of the nine women were measured in the same phase of their menstrual cycle. Only three women were measured in different periods of the cycle. With regard to these women, it is unlikely that intake of savoury fat was affected by the phase of the menstrual cycle. The % energy as fat was much higher than usual, and chocolate was not part of it.

We conclude that oral administration of ENT (45 mg/d) together with a high-fat diet did not affect food intake, appetite, EE or body weight in subjects with a preference for a high-fat diet experiencing a negative energy and fat balance.

References

Bowyer RC, Rowston WM, Jehanli AMT, Lacey JH & Hermon-Taylor J (1993) The effect of satiating meal on the concentrations of procolipase activation peptide in the serum and urine of normal and morbidity obese individuals. *Gut* **34**, 1520–1525.

Bray GA (2000) Afferent signals regulating food intake. *Proc Nutr Soc* **59**, 373–384.

Brouwer E (1957) On simple formulae for calculating the heat expenditure and the quantities of carbohydrate and fat oxidised in metabolism of men and animals, from gaseous exchange (oxygen intake and carbonic output) and urine-N. *Acta Physiol Pharmacol N* **6**, 795–802.

Erlanson-Albertsson C, Mei J, Okada S, York D & Bray GA (1991) Pancreatic procolipase propeptide, enterostatin, specifically inhibits fat intake. *Physiol Behav* **49**, 1191–1194.

Erlanson-Albertsson C & York D (1997) Enterostatin – A peptide regulating fat intake. *Obes Res* **5**, 360–372.

Harris JA & Benedict FG (1919) *A Biometric Study of Basal Metabolism in Man*. Washington: Carnegie Institution.

Herman CP & Polivy J (1980) Restrained eating. In *Obesity*, pp. 208–225 [AJ Stunkard, editor]. Philadelphia: W. B. Saunders.

Jas PE (1996) Changes in food intake and mood across the menstrual cycle. PhD Thesis, University of Reading.

Lin L, Chen J & York DA (1997) Chronic icv enterostatin preferentially reduced fat intake and lowered body weight. *Peptides* **18**, 657–661.

Lin L & York DA (1994) Metergoline blocks the feeding response to enterostatin. *Appetite* **23**, 313 Abstr.

Lin L & York DA (1998) Chronic ingestion of dietary fat is a prerequisite for inhibition of feeding by enterostatin. *Am J Physiol* **275**, R619–R623.

Lorr M & McNair DM (1984) *Manual. Prile Mood States. Bi-Polar Form (POMS-BI)*. San Diego, CA: Educational and Industrial Testing Service (EDITS).

Mei J, Cheng Y & Erlanson-Albertsson C (1993) Enterostatin – its ability to inhibit insulin secretion and to decrease high-fat food intake. *Int J Obes Relat Metabol Disord* **17**, 701–704.

Mei J & Erlanson-Albertsson C (1996) Plasma insulin response to enterostatin and effect of adrenalectomy in rat. *Obes Res* **4**, 513–519.

Nagase H, Bray GA & York DA (1997) Effect of galanin and enterostatin on sympathetic nerve activity to interscapular brown adipose tissue. *Brain Res* **709**, 44–50.

Okada S, York DA, Bray GA & Erlanson-Albertsson C (1991) Enterostatin (Val–Pro–Asp–Pro–Arg) the activation peptide of procolipase selectively reduces fat intake. *Physiol Behav* **49**, 1185–1189.

Okada S, York DA, Bray GA, Mei J & Erlanson-Albertsson C (1992) Differential inhibition of fat intake in two strains of rat by the peptide enterostatin. *Am J Clin Nutr* **262**, R1111–R1116.

Okada S, Lin L, York DA & Bray GA (1993) Chronic effects of intracerebral ventricular enterostatin in Osborne–Mendel rats fed a high-fat diet. *Physiol Behav* **54**, 325–330.

Ookuma KC, Barton C, York DA & Bray GA (1997) Effect of enterostatin and kappa-opioids on macronutrient selection and consumption. *Peptides* **18**, 785–791.

Prasad C, Imamura M, Debata C, Svec F, Sumar N & Hermon-Taylor J (1999) Hyperenterostatinemia in premenopausal obese women. *J Clin Endocrinol Metabol* **84**, 937–941.

Rössner S, Barkeling B, Erlanson-Albertsson C, Larsson P & Wahlin-Boll E (1995) Intravenous enterostatin does not affect single meal food intake in man. *Appetite* **24**, 37–42.

Rozin P, Levine E & Stoess C (1991) Chocolate craving and liking. *Appetite* **17**, 199–212.

Schoffelen PFM, Westerterp KR, Saris WHM & ten Hoor F (1997) A dual respiration chamber with automated calibration. *J Appl Physiol* **83**, 2064–2072.

Schrauwen P, Marken Lichtenbelt van WD, Saris WHM & Westerterp KR (1997) Changes in nutrient oxidation in response to a high-fat diet. *Am J Clin Nutr* **66**, 276–282.

Schutz Y (1995) Macronutrients and energy balance in obesity. *Metabolism* **44**, Suppl., 7–11.

Smeets M, Geiselman P, Bray GA & York DA (1999) The effect of oral enterostatin on hunger and food intake in human volunteers. *FASEB J* **13**, A871 Abstr.

Sörhede M, Mei J & Erlanson-Albertsson C (1993) Enterostatin – a gut-brain peptide – regulating fat intake in rat. *J Physiol Paris* **87**, 273–275.

Stichting Nederlands Voedingsstoffenbestand (1996) *Nevo Tabel (New Tables)*. Den Haag: Voorlichtingsbureau voor de voeding.

Stunkard AJ & Messick S (1985) The three-factor eating questionnaire to measure dietary restraint, disinhibition, and hunger. *J Psychosom Res* **29**, 71–83.

Swinburn BA & Ravusssin E (1993) Energy balance or fat balance? *Am J Clin Nutr* **57**, Suppl., 766S–770S.

Tarasuk V & Beaton GH (1991) Menstrual-cycle patterns in energy and macronutrient intake. *Am J Clin Nutr* **53**, 442–447.

Tian Q, Nagase H, York DA & Bray GA (1994) Vagal-central nervous system interactions modulate the feeding response to peripheral enterostatin. *Obes Res* **2**, 527–534.

Tomelleri R & Grunewald K (1987) Menstrual cycle and food cravings in young college women. *J Am Med Assoc* **87**, 829–837.

Weir JBdeV (1949) New methods for calculating metabolic rate with special references to protein metabolism. *J Physiol* **109**, 1–9.

Westerterp KR, Wilson SAJ & Rolland V (1998) Diet induced thermogenesis measured over 24 h in a respiration chamber: effect of diet composition. *Int J Obes Relat Metabol Disord* **22**, 1–6.

Westerterp-Plantenga MS, Rolland V, Wilson SAJ & Westerterp KR (1999) Satiety related to 24 h diet-induced thermogenesis during high protein/carbohydrate vs high fat diets measured in a respiration chamber. *Eur J Clin Nutr* **53**, 1–8.